[Frontiers in Bioscience 8, s410-420, May 1, 2003]

THE INSULIN-REGULATED AMINOPEPTIDASE: A COMPANION AND REGULATOR OF GLUT4

Susanna R. Keller

University of Virginia, Department of Internal Medicine-Division of Endocrinology, PO Box 801409, Charlottesville VA 22908

TABLE OF CONTENTS

1. ABSTRACT

The insulin-regulated membrane aminopeptidase (IRAP) was originally identified in fat and muscle cells as a major protein in intracellular vesicles that also harbor the insulin-responsive glucose transporter GLUT4. IRAP, like GLUT4, predominantly localizes to these intracellular vesicles under basal conditions. In response to insulin IRAP, like GLUT4, translocates to the plasma membrane. Purification and cloning of IRAP revealed that it was a novel member of the family of zinc-dependent membrane aminopeptidases. Upon the cloning of the human placental oxytocinase (P-LAP) it was discovered that IRAP and P-LAP were the rat and human homologues of the same protein. The expression of IRAP/P-LAP is not limited to fat and muscle cells, and the subcellular distribution of IRAP/P-LAP is regulated by different peptide hormones and exercise. IRAP/P-LAP cleaves several peptide hormones in vitro. In insulin- and oxytocin-treated cells, concomitant with the appearance of IRAP/P-LAP at the cell surface, aminopeptidase activity toward extracellular substrates increases. A physiological function for IRAP/P-LAP may thus be the processing of circulating peptide hormones. These extracellular substrates, however, would be processed efficiently only when IRAP/P-LAP gets access to them after translocation to the cell surface upon stimulation of cells with insulin or other factors. The in vivo substrates for IRAP/P-LAP remain to be determined. The initial characterization of mice in which IRAP/P-LAP was deleted (IRAP -/- mice) revealed that GLUT4 protein levels were dramatically decreased in all fat and muscle tissues. This finding suggests a function for IRAP/P-LAP in the regulation of GLUT4 levels. Further characterization of the IRAP -/- mice is required to elucidate the role IRAP/P-LAP may play in the control of peptide hormone metabolism.

2. INTRODUCTION

The insulin-regulated membrane aminopeptidase (IRAP) was discovered as a major protein in intracellular vesicles that harbor the insulin-responsive glucose transporter isotype GLUT4 (1-3). These vesicles, mostly referred to as GLUT4 vesicles, have been extensively characterized (4-10). They are small vesicles that are isolated from the low density microsomes of fat and muscle cells by affinity purification with antibodies directed against the C-terminal cytoplasmic peptide of GLUT4. The analysis of their protein composition revealed that they represent the different cellular compartments through which GLUT4 trafficks (4-10). What made IRAP interesting and unique among the GLUT4 vesicle proteins that have been characterized so far was that it behaved like GLUT4 (1,2). It was not only sequestered in the same intracellular vesicles as GLUT4 under basal conditions, it also redistributed to the cell surface after stimulation of cells with insulin.

At the time of its discovery IRAP was referred to as vp165 for vesicle protein of 165 kDa (1) or gp160 for glycoprotein of 160 kDa (2). The purification of IRAP (1,2,11) and subsequent cloning of its cDNA (12) revealed that it was an aminopeptidase and it was renamed insulin-regulated aminopeptidase (IRAP). Upon the cloning of the human oxytocinase/placental leucine aminopeptidase (P-LAP) it became evident that IRAP and P-LAP were the rat and human homolog of the same protein (13). They are 87% identical at the amino acid level. For the remainder of this review I will therefore use the term IRAP/P-LAP.

IRAP/P-LAP is a type II membrane protein with an N-terminal 109 amino acid cytoplasmic tail, a single transmembrane segment, and a large extracellular/intralumenal domain (894 amino acids) (12). The cytoplasmic tail contains motifs that are responsible for its specific subcellular distribution and regulation by insulin (Figure 1)(12). The central portion of the extracellular/intralumenal domain contains the sites that are

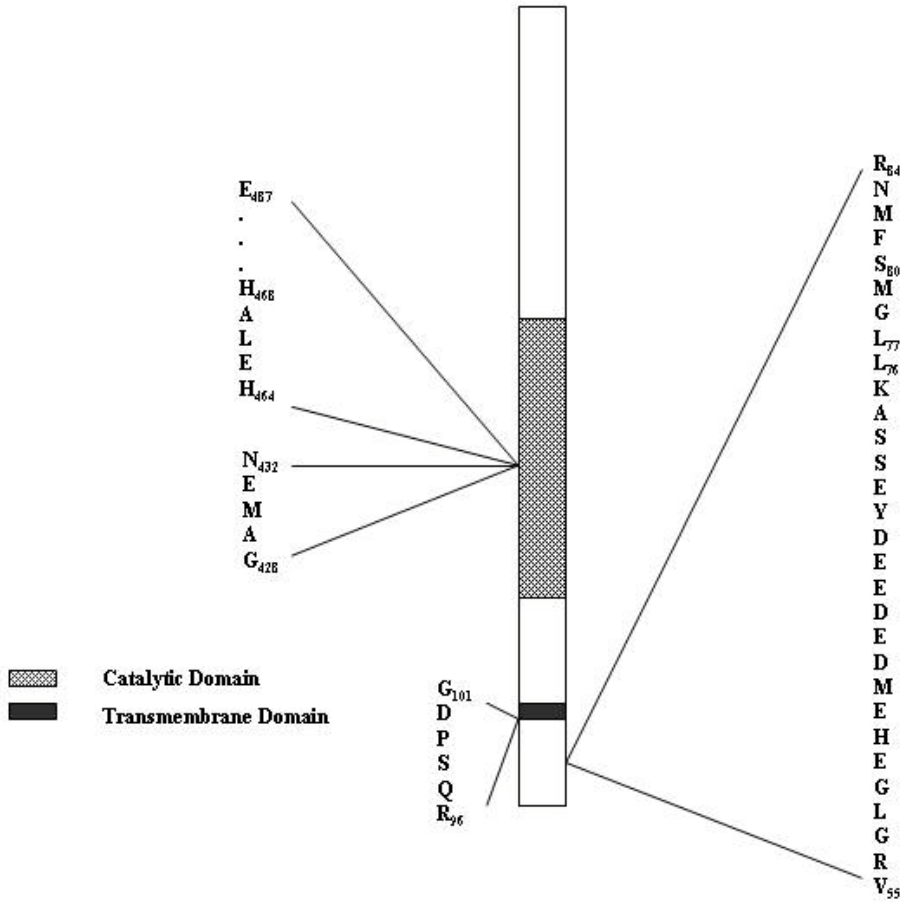

Figure 1. Domain structure of IRAP/P-LAP. Highlighted are the sequences in the cytoplasmic tail that are crucial for the subcellular localization of IRAP (55-84) and that contain the dileucine sequence 76/77 preceeded by a cluster of acidic amino acids. In addition, shown is the tankyrase interaction motif (96-101). In the extracellular/intralumenal domain the motifs that are essential for catalytic function, the characteristic zinc-binding motif (aa 464-468 and aa 487) and the GAMEN motif (aa 428-32), are shown.

essential for catalytic function and define it as a member of the family of zinc-dependent aminopeptidases (Figure 1) (12,14,15). The theoretical Mr for IRAP/P-LAP is 117,317. IRAP/P-LAP is heavily glycosylated and the molecular mass of the processed protein is therefore larger, 165 kDa in most tissues and 140 kDa in the brain (12).

In the following I will first review studies on the characterization of the subcellular localization of IRAP/P-LAP and its regulation as a companion of GLUT4. Second, I will summarize studies that address the question of the physiological function of IRAP/P-LAP.

3. EXPRESSION AND SUBCELLULAR LOCALIZATION OF IRAP/P-LAP

3.1. Expression and subcellular localization in fat and muscle cells

IRAP/P-LAP is well expressed in white and brown adipocytes, cardiomyocytes and skeletal muscle cells (12), as well as in adipocyte and muscle cell lines, 3T3-L1 adipocytes and L6 myotubes (16,17). In these cell types IRAP/P-LAP has been extensively characterized with regard to its subcellular localization and the regulation of its trafficking by insulin as a companion of GLUT4 (1,2,16-20). Using subcellular fractionation and immunadsorption of intracellular vesicles followed by immunoblotting, as well as immunohistochemistry and -electron microscopy it has been demonstrated that IRAP/P-LAP and GLUT4 colocalize to the same intracellular vesicular compartments and the plasma membrane. There is only one exception to this rule; in atrial cardiomyocytes a large proportion of GLUT4 was also present in secretory granules containing atrial natriuretic factor, whereas IRAP/P-LAP was not detected in these granules (20).

Furthermore, it was observed that the relative distributions between the intracellular compartments and the plasma membrane under basal and insulin-stimulated conditions were the same for IRAP/P-LAP and GLUT4. Under basal conditions more than 90% of IRAP/P-LAP and GLUT4 are found within the cells (21-24). Within five minutes of exposure of cells to a maximal dose of insulin 50% of IRAP/P-LAP and GLUT4 are found at the cell

surface (21,22,24-26). The relative increases of IRAP/P-LAP and GLUT4 at the cell surface of adipocytes were shown to be 8 and 10-20 fold, respectively (16,24,27).

The kinetics of the trafficking of IRAP/P-LAP through the different subcellular compartments under basal and insulin-stimulated conditions have been determined in 3T3-L1 adipocytes using a fusion protein of the cytoplasmic domain of IRAP/P-LAP with the transmembrane and extracellular domains of the transferrin receptor (23). The studies demonstrated that under basal conditions the fusion protein is externalized very slowly and is efficiently removed from the cell surface. This thus leads to the accumulation of the fusion protein within the cells and an almost complete exclusion from the cell surface. Insulin stimulation of cells increases the externalization rate constant of the fusion protein dramatically while having little effect on its internalization rate constant. This results in the redistribution of the fusion protein to the plasma membrane. The kinetic parameters determined for the IRAP/P-LAP-transferrin receptor fusion protein are similar to the ones that had been determined earlier for labelled endogenous GLUT4 in 3T3-L1 adipocytes (26).

IRAP/P-LAP and GLUT4 are so far the only known molecules in fat and muscle cells that exhibit such an efficient intracellular sequestration in non-stimulated cells and marked translocation to the cell surface in response to insulin. Insulin stimulates the redistribution of the glucose transporter isoform GLUT1, the transferrin receptor, and the mannose-6-phosphate/IGF-II receptor to the cell surface in various cell types including fat cells (23,28,29). However, these proteins are present at the cell surface to a considerable extent under basal conditions, and the magnitude of the insulin-stimulated increase is only 2-3 fold.

The recruitment of IRAP/P-LAP and GLUT4 to the cell surface of fat and muscle cells is not restricted to insulin action. It has been shown that endothelin-1 leads to the translocation of IRAP/P-LAP and GLUT4 to the plasma membrane in 3T3-L1 adipocytes and neonatal cardiomyocytes (30,31). In skeletal muscle acute exercise elicits the redistribution of IRAP/P-LAP and GLUT4 to the cell surface (32) and endurance exercise training leads to an increased expression of IRAP/P-LAP and GLUT4 (33).

The precise nature of the intracellular compartment(s) where IRAP/P-LAP and GLUT4 predominantly reside is still under investigation. By immunohistochemistry and -electron microscopy IRAP/P-LAP and GLUT4 were localized to the trans-Golgi reticulum and to tubulo-vesicular structures dispersed in the cytoplasm (20). Biochemical characterization showed that in both adipocytes and myocytes two major distinct intracellular pools each containing approximately 50% of IRAP/P-LAP and GLUT4 could be distinguished (19,20). One of the two pools is marked by the presence of the transferrin receptor and thus represents the general recycling endosomes. The second pool is regarded as a specialized compartment from which the transferrin receptor is excluded. A recent study in 3T3-L1 adipocytes showed with a compartment-specific fluorescence-quenching assay that these two pools are in dynamic communication with one another and with the cell surface (34). Based on the results from this study the following model was proposed: IRAP/P-LAP and GLUT4 are retained within the transferrin-containing endosomal recycling compartment. From this compartment vesicles containing selectively IRAP/P-LAP and GLUT4 bud and join a specialized postendosomal pool of vesicles. Vesicles from the latter pool move to and fuse with the cell surface. Under both basal and insulin-stimulated conditions IRAP/P-LAP and GLUT4 continuously recycle between these two compartments and the cell surface. However, under basal conditions the budding of IRAP/P-LAP and GLUT4-containing vesicles from the endosomal recycling compartment and their movement to the cell surface through the postendosomal compartment are slow. Insulin stimulates the recruitment of IRAP/P-LAP and GLUT4 to the cell surface through the regulation of the formation and movement of IRAP/P-LAP and GLUT4-containing vesicles from both intracellular pools (Figure 2) (34).

3.2. Expression and subcellular localization of IRAP/P-LAP in other cell types

The studies in fat and muscle cells summarized above showed that IRAP/P-LAP behaves exactly like GLUT4 with regard to its sucellular localization and regulation by various stimuli. However, in contrast to GLUT4, IRAP/P-LAP is not only found in muscle and fat cells, but is expressed in all major tissues (12,13,35,36). Immunohistochemical analysis showed that in different tissues the expression of IRAP/P-LAP is restricted to specific cell types (35). IRAP/P-LAP has so far been detected in vascular endothelial cells, gastrointestinal mucosal cells, epithelial cells of the hepato-biliary, pancreato-biliary, bronchial-alveolar and renal tubular systems, islet cells of the pancreas (35), neuronal cells in the central nervous system (35,36), syncytiotrophoblasts of the placenta (35,37), endometrial epithelial cells (38), sweat-gland cells, seminal vesicles and prostate gland (35). In neuronal cells the staining was described as diffuse intracellular and in basal ganglion neurons as punctate intracellular (36). In syncytiotrophoblasts apical membrane staining (37) was observed, and in endometrial endothelial cells IRAP/P-LAP was localized to the plasma membrane and to cytoplasmic granules (38).

The subcellular localization and trafficking characteristics of IRAP/P-LAP have been evaluated extensively in Chinese hamster ovary cells (CHO), a cell line that expresses IRAP/P-LAP, but not GLUT4 (39). In these studies again the fusion protein between the cytoplasmic domain of IRAP/P-LAP and the transmembrane and extracellular domains of the transferrin receptor was used. As in 3T3-L1 adipocytes the fusion protein was only slowly recycled to the cell surface under basal conditions, and insulin elicited an increase in the externalization rate constant of the fusion protein. However, the increase in the externalization rate constant was smaller in the CHO cells than in the 3T3-L1 adipocytes, 2-3 versus 4-5 fold, respectively (23,39). Later

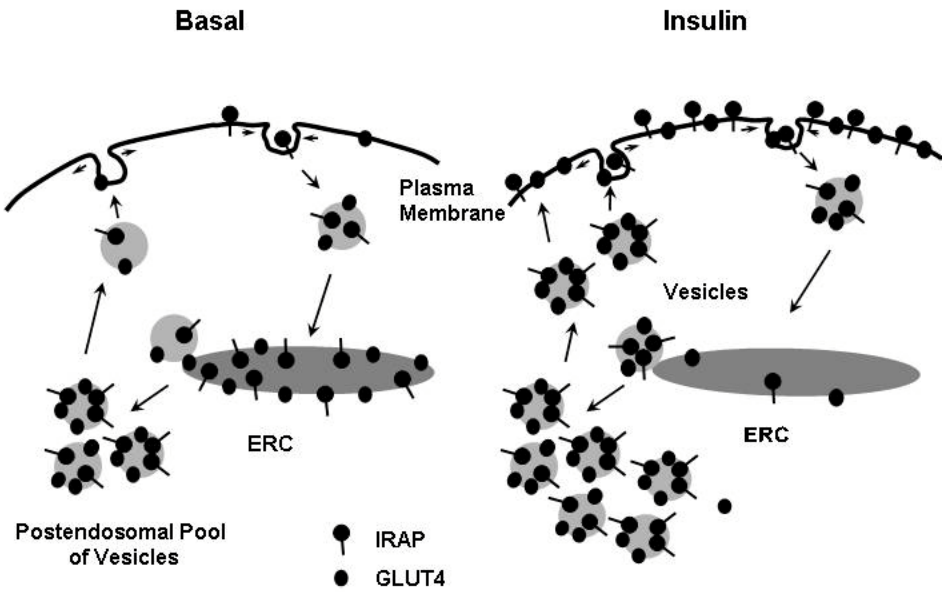

Figure 2. Subcellular distribution of IRAP/P-LAP and GLUT4 under basal and insulin-stimulated conditions. ERC = endosomal recycling compartment.

studies revealed that the intracellular compartments in which IRAP/P-LAP is retained in the CHO cells and the 3T3-L1 adipocytes are different (40). In CHO cells the subcellular localization of the IRAP/P-LAP fusion protein and the endogenous IRAP/P-LAP is identical to the wild type transferrin receptor. Thus, the entire pool of IRAP/P-LAP localizes to the general endosomal recycling compartment. A GLUT4 construct that was also introduced into the CHO cells showed the same behavior as the IRAP/P-LAP transferrin receptor fusion protein (40).

IRAP/P-LAP is expressed in undifferentiated 3T3-L1 fibroblasts albeit at levels that are about 6-fold lower than in differentiated 3T3-L1 adipocytes (16). GLUT4 is only expressed upon differentiation of the fibroblasts into adipocytes (16). The finding of the expression of IRAP/P-LAP in the fibroblasts provided an opportunity to compare its subcellular distribution and trafficking between fibroblasts and adipocytes. The trafficking kinetics for the fusion protein of the cytoplasmic domain of IRAP/P-LAP and the transmembrane and extracellular domains of the transferrin receptor in the 3T3-L1 fibroblasts were similar to the ones described above for CHO cells (40). Using a cell surface labeling method it was found that under basal conditions the relative amount of IRAP/P-LAP at the cell surface was about three times higher in undifferentiated fibroblasts than in differentiated adipocytes (25). After insulin treatment the proportion of IRAP/P-LAP at the cell surface was the same for both cell types. Cell surface expression for the transferrin receptor under basal and insulin-stimulated conditions were almost identical between the fibroblasts and adipocytes. These findings implied that the sequestration of IRAP/P-LAP is enhanced during differentiation and that this may be the basis for the larger insulin-elicited fold increase of IRAP/P-LAP at the cell surface of adipocytes. It was suggested that the expression of GLUT4 may be important in the establishment of the sequestration mechanisms during differentiation. However, this is not the case. It was shown that the retention of IRAP/P-LAP within the differentiating cells preceeds the expression of GLUT4 (41).

IRAP/P-LAP is also expressed in rat pheochromocytoma PC12 cells (36,42). Its levels increase by 2.5 fold when cells are differentiated into neuronal cells by the addition of neuronal growth factor (36). By immunohistochemistry IRAP/P-LAP was detected in a granular pattern within the cells in the cell bodies as well as in the neurites (36,42). Further characterization of the IRAP-containing intracellular compartment by gradient centrifugation and immunoadsorption revealed that IRAP/P-LAP resided in a population of vesicles that are different from small synaptic vesicles and secretory granules (42). Cell surface labeling showed that IRAP/P-LAP was expressed at the plasma membrane under basal conditions (36,42). Treatment of cells with forskolin led to a small (30%)(36) or no increase (42) of IRAP/P-LAP at the cell surface. Insulin stimulation or depolarization of cells did not lead to the translocation of IRAP/P-LAP to the plasma membrane (42).

IRAP/P-LAP has also been characterized in primary human umbilical vascular endothelial (HUVEC) cells (43). Cell surface expression of IRAP/P-LAP was determined under basal conditions and after stimulation of cells with oxytocin by cell surface labeling and by measuring

aminopeptidase activity toward an extracellular substrate. It was found that oxytocin led to a 3-4 fold increase of IRAP/P-LAP at the cell surface. These studies thus showed that in HUVEC cells IRAP/P-LAP is also sequestered within the cells and that oxytocin changed its subcellular distribution.

3.3. Trafficking motifs in the IRAP/P-LAP cytoplasmic domain

The molecular mechanisms that are responsible for the intracellular sequestration of IRAP/P-LAP and GLUT4 and the insulin-triggered translocation to the cell surface are subjects of intensive investigations. For IRAP/P-LAP, we have been able to show that the cytoplasmic tail carries all the information for its specific intracellular localization and insulin-regulated trafficking (23,39). Extensive studies with the three major cytoplasmic domains in GLUT4, the N-terminus, the cytoplasmic loop between membrane-spanning domains 6 and 7, and the C-terminus have identified roles for each of these in GLUT4 trafficking (44).

The cytoplasmic tail of IRAP/P-LAP contains several potential trafficking motifs (two dileucine motifs and two clusters of acidic amino acids) similar to those found in the C-terminal cytoplasmic tail of GLUT4 (12). Site-directed mutagenesis revealed that the dileucine sequence at position 76/77, but not the dileucine sequence at position 53/54, in the cytoplasmic tail of IRAP/P-LAP, was required for intracellular retention in 3T3-L1 adipocytes and CHO fibroblasts (23,39). Further analysis of mutants of the cytoplasmic tail of IRAP/P-LAP has only been carried out in CHO fibroblasts. These studies showed that a 29 amino acid sequence (residues 56-84) that includes the dileucine motif at position 76/77 preceeded by a cluster of acidic amino acids (Figure 1) was necessary and sufficient for the dynamic retention of IRAP/P-LAP in the endosomal recycling compartment (45). The serine at position 80 is phosphorylated by protein kinase C zeta in vitro (46). It remains to be established whether this site is phosphorylated in vivo in response to insulin. Protein kinase C zeta is activated upon stimulation of cells with insulin, and it is therefore possible that serine phosphorylation close to the dileucine sequence 76/77 may lead to the release of IRAP/P-LAP from sequestration.

In GLUT4 the dileucine sequence at position 489/490 functions as an internalization and a sequestration motif (44,47). The acidic cluster TELEYLGP (498-505) is important in targeting GLUT4 to the postendosomal specialized compartment in 3T3-L1 adipocytes (48). Earlier studies on GLUT4 had identified serine 488 in the C-terminal cytoplasmic domain of GLUT4 as a site for phosphorylation by cAMP-dependent protein kinase in vitro. (49). The site was phosphorylated in response to isoproterenol in vivo, but was not phosphorylated upon stimulation with insulin. Mutation of the site did not affect sequestration and insulin-stimulated relocation of GLUT4 to the cell surface (50).

3.4. Proteins interacting with the cytoplasmic tail of IRAP/P-LAP

Sorting and retention of IRAP/P-LAP and GLUT4 most likely require interactions of the cytoplasmic domains of IRAP/P-LAP and GLUT4 with other proteins. Since injection of either a GST fusion protein with the cytoplasmic tail of IRAP/P-LAP or a peptide derived from the C-terminal end of GLUT4 trigger the redistribution of both IRAP/P-LAP and GLUT4 to the cell surface (51,52) it is very likely that the same mechanisms are responsible for their sequestration within the cells under basal conditions. A strong physical interaction between IRAP/P-LAP and GLUT4 has been ruled out; no IRAP/P-LAP is found in immunoprecipitates derived from detergent cell lysates using an antibody against GLUT4 and vice versa (16).

Several attempts have been made to find proteins that interact with the cytoplasmic domain of IRAP/P-LAP. So far tankyrase-1 and tankyrase-2 and long-chain, medium-chain and short-chain acyl-coenzyme A dehydrogenases have been identified (53-55).

Tankyrases are ADP-ribose transferases that were originally characterized as telomeric proteins. After the discovery of the interaction of tankyrases with the IRAP/P-LAP cytoplasmic domain it was shown that tankyrases colocalize with GLUT4 in the juxtanuclear region of 3T3-L1 adipocytes (53). The interaction of tankyrases with IRAP/P-LAP involves the ankyrin-repeat domain in the tankyrases and the sequence RQSPDG (96-101) in the IRAP/P-LAP cytoplasmic tail (Figure 1). Insulin induces the phosphorylation of tankyrase through the mitogen-activated protein kinase pathway and stimulates its poly (ADP-ribose) polymerase activity. However, tankyrases do not seem to play a role in the acute effect of insulin on GLUT4 translocation, and Chi & Lodish speculated that tankyrases may be involved in long term regulation of the metabolism of GLUT4 vesicles (53).

The acyl-coenzyme A dehydrogenases (ACDs) interact with the cytoplasmic sequence 55-82 in IRAP/P-LAP in both adipocytes and skeletal muscle (55). The interaction is dependent on the dileucine sequence 76/77 in IRAP/P-LAP and ACD inhibitors disrupt the interaction *in vitro*. Since inhibitors, when applied to permeabilized 3T3-L1 adipocytes, led to a translocation of GLUT4 to the cell surface it was hypothesized that the ACDs may be involved in the anchoring of GLUT4 vesicles to an intracellular compartment via association with IRAP/P-LAP (55).

The tankyrases and the ACDs do not bind to the C-terminal cytoplasmic sequence of GLUT4 (53,55). However, a L-3-hydroxyacyl-CoA dehydrogenase has been shown to interact with the C-terminal sequence of GLUT4 (56).

In conclusion, the proteins that are crucial for the sorting and retention of IRAP/P-LAP and GLUT4 remain to be discovered.

4. PHYSIOLOGICAL FUNCTION OF IRAP/P-LAP

The physiological consequence of the differential subcellular distribution of GLUT4 in basal and insulin-stimulated cells is well-established (57). When little GLUT4 is at the cell surface under basal conditions the uptake of glucose into fat and muscle cells is low. When in

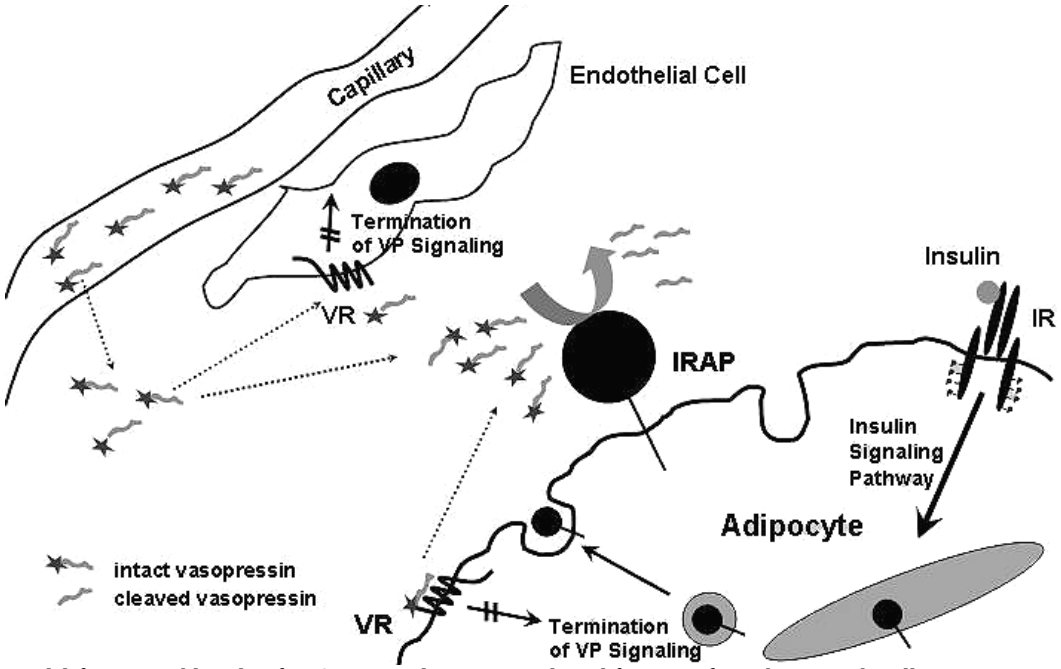

Figure 3. Model for a possible role of IRAP in insulin action and modification of, as shown in this illustration as an example, vasopressin action. IR = insulin receptor, VR = vasopressin receptor.

response to insulin the number of GLUT4 increases at the cell surface glucose uptake is maximally stimulated. This effect is responsible for the disposal of 80-90% of glucose after a meal and a key mechanism in maintaining glucose homeostasis. The question is what the physiological function of IRAP/P-LAP is and what role it plays in insulin action.

The membrane aminopeptidases A and N and the thyrotropin-releasing hormone degrading enzyme have been shown to process regulatory peptides, thereby changing their activities (58-61). These precedents suggested that IRAP/P-LAP also processes one or several regulatory peptides. Indeed it had been shown earlier with purified P-LAP that it cleaved regulatory peptides (61,62).

4.1. Peptide hormones cleaved by IRAP/P-LAP

IRAP/P-LAP cleaves vasopressin, oxytocin, lys-bradykinin, angiotensins III and IV, and the neuropeptides met-enkephalin and dynorphin A(1-8) in vitro (36,62,63). Other peptide hormones, among these insulin, have been tested for cleavage by IRAP/P-LAP and found to be unlikely substrates for IRAP/P-LAP (63,64). The cleavage of the N-terminal amino acid from each angiotensin converts it from one active form to another active form (65). In the case of vasopressin and oxytocin the cleavage of the N-terminal cysteine inactivates the peptide hormone (66). The effect of the conversion of lys-bradykinin to bradykinin is unknown; the two peptides do not show any differences in their action (67). Met-enkephalin and dynorphin A are inactivated by the cleavage of their amino-terminal amino acids (36,68).

Angiotensins III and IV and met-enkephalin are also efficiently cleaved by aminopeptidase N *in vitro* (58). However, IRAP/P-LAP is the only membrane aminopeptidase known to cleave vasopressin and oxytocin, hormones with an N-terminal cystine (66). Whether these peptide hormones are also *in vivo* substrates for IRAP/P-LAP remains to be determined. The Km's of these peptides for cleavage by IRAP/P-LAP *in vitro* are in the 10-1000 nanomolar range (63), whereas these peptide hormones are present at picomolar concentrations in the circulation. However, it is possible that local concentrations of the hormones in the tissues are higher or that the affinities of the hormones for IRAP/P-LAP are larger under physiological conditions. These peptide hormones may thus be cleaved efficiently by IRAP/P-LAP *in vivo.*

Recently IRAP/P-LAP has been identified as the AT4 receptor (69). AT4 not only binds to IRAP/P-LAP, but also inhibits the activity of IRAP/P-LAP (63,69). It remains to be detemined what the physiological role of the interaction of AT4 with IRAP/P-LAP is.

4.2. Role of IRAP/P-LAP in insulin action

The membrane aminopeptidases A and N are constitutively expressed at the cell surface and so have continued access to their extracellular substrates (70). As described above IRAP/P-LAP is sequestered within the cells under basal conditions and is only present in high numbers at the cell surface of adipocytes and muscle cells after treatment of cells with insulin. A possible role of IRAP/P-LAP in insulin action could thus be envisioned as following (Figure 3). Insulin through activation of its own cell surface receptor elicits the translocation of IRAP/P-LAP to the cell surface. IRAP/P-LAP consequently gains access to peptide hormones present in the extracellular milieu and removes the N-terminal amino acids of suitable substrates. When vasopressin or oxytocin, two likely

specific IRAP/P-LAP substrates, are processed by IRAP/P-LAP they are inactivated and consequently signaling from vasopressin and oxytocin receptors is terminated. Depending on where the specific receptors localize, vasopressin and oxytocin action in the same cells on which insulin exerts its effects or in neighboring cells in which insulin causes no effects, is modified. This proposed function for IRAP/P-LAP opens up the potential that insulin can modify the action of other peptide hormones and thus broaden its own spectrum of action. This concept may not be restricted to the action of insulin since other peptide hormones can also lead to the translocation of IRAP/P-LAP to the cell surface (30,31,43). It also may not be restricted to fat and muscle tissues since as described above IRAP/P-LAP is expressed in all major tissues so far tested (12,13,35,36).

In support of this model we have found that the cleavage of vasopressin by isolated adipocytes is indeed increased 3-fold by insulin concomitant with the increase of IRAP/P-LAP at the cell surface (63). Also, as described above, Nakamura et al. showed that in vascular endothelial cells oxytocin elicits the translocation of IRAP-P-LAP to the cell surface and simultaneously stimulates aminopeptidase activity toward an extracellular synthetic substrate for IRAP/P-LAP (43).

4.3. Characterization of IRAP/P-LAP knockout mice

To address the question of the function of IRAP/P-LAP in a physiological context we have generated mice with a targeted disruption of the IRAP/P-LAP gene (referred to as IRAP -/- mice). These mice were first tested for defects in the regulation of glucose homeostasis, a major insulin action that is dependent on the proper regulation of GLUT4 (71).

One of the most important findings in the initial characterization of the IRAP -/- mice was that the total amounts of GLUT4 protein were diminished by 50-80% in the IRAP -/- mice in all the tissues where GLUT4 is predominantly expressed, in skeletal muscle, heart, and adipose tissues (71). The decreases for GLUT4 were similar in the tissues from male and female IRAP -/- mice and were present as early as postnatal day 18. These findings thus suggest that the presence of IRAP/P-LAP is required for the maintenance of normal GLUT4 levels independent of the tissue type, sex and age. The mechanisms responsible for the decrease of GLUT4 are currently under investigation. One explanation for the decrease in GLUT4 in the different tissues is that changes in the action of peptide hormones that are substrates for IRAP/P-LAP lead to a decrease in the synthesis or an increase in the degradation of GLUT4. Alternatively, since GLUT4 and IRAP/P-LAP colocalize to the same intracellular vesicles in muscle and fat cells, the absence of IRAP/P-LAP could compromise the sorting and trafficking of GLUT4 and lead to an increased degradation. With regard to the latter possibility we found that under steady state conditions the relative distribution of GLUT4 in subcellular fractions of basal and insulin-stimulated IRAP -/- adipocytes was the same as in control cells (71).

Despite the dramatic decreases in GLUT4 expression glucose homeostasis was maintained in the IRAP -/- mice. This is in sharp contrast to genetically modified mouse models in which GLUT4 expression was reduced (reviewed in 71). The minimal effect of the substantial decrease of GLUT4 in skeletal muscle on glucose homeostasis in the IRAP -/- mice may be explained by the fact that glucose uptake into skeletal muscles was not severely impaired. Studies are in progress to determine how the IRAP -/- mice compensate for the defect in GLUT4 and whether another isotype of the growing family of glucose transporters is upregulated (72).

The finding of the decreased expression of GLUT4 in the IRAP -/- mice is parallelled by the finding of a decreased expression of IRAP/P-LAP in GLUT4 -/- mice (73). However, the finding of the normal relative subcellular distribution of GLUT4 in the IRAP -/- adipocytes contrasts with the finding of an abnormal subcellular distribution for IRAP/P-LAP described in GLUT4 -/- adipocytes. In the latter, IRAP/P-LAP is redistributed to the plasma membrane under basal conditions and there is no further increase in IRAP/P-LAP in this fraction in response to insulin (73).

The initial characterization of the IRAP -/- mice revealed another interesting finding, an increase in heart size (71). In all the mouse models with decreased or lack of GLUT4 expression cardiac hypertrophies have been observed (reviewed in 71). Whether in the IRAP -/- mice the cardiac abnormality is due to the decrease in GLUT4 expression or whether it is a consequence of the impaired processing of a peptide hormone which is a substrate for IRAP/P-LAP remains to be determined. It is noteworthy that several of the peptide hormones found to be substrates for IRAP/P-LAP in vitro are vasoactive peptide hormones (63). If one of these is an in vivo substrate for IRAP/P-LAP and is not processed properly in the IRAP -/- mice cardiovascular abnormalities may develop.

5. PERSPECTIVE

The findings in the IRAP -/- mice provide strong evidence for a role of IRAP/P-LAP in the regulation of GLUT4 levels. Thus, in fat and muscle cells IRAP/P-LAP may not only be a companion, but also a regulator of GLUT4. Further characterization of the IRAP -/- mice is required to determine the molecular mechanisms through which IRAP/P-LAP modifies GLUT4 expression.

Similar to GLUT4, the recruitment of IRAP/P-LAP to the cell surface is impaired in type 2 diabetics (74,75) and consequently it would be expected that IRAP/P-LAP action at the cell surface is diminished in these individuals. Type 2 diabetics show decreased expression of GLUT4 in adipose tissues (57) and the development of cardiovascular complications in diabetics is common (76). The changes we observed in the initial characterization of the IRAP -/- mice, decreased expression of GLUT4 and the enlargement of the heart, suggest that impaired function of IRAP/P-LAP at the cell surface may play a role in the development of complications in insulin-resistant individuals.

Further analysis of the regulation of peptide hormone metabolism and action in the IRAP -/- mice is required to gain an understanding of the physiological function of IRAP/P-LAP and the role it plays in insulin action.

6. ACKNOWLEDGMENTS

Part of the research reviewed in here was supported by grants from the National Institutes of Health Grants DK 25336 and DK58051.

7. REFERENCES

1. C.C. Mastick, R. Aebersold & G.E. Lienhard: Characterization of a major protein in GLUT4 vesicles. Concentration in the vesicles and insulin-stimulated translocation to the plasma membrane. *J Biol Chem* 269, 6089-6092 (1994)

2. K. Kandror & P.F. Pilch: Identification and isolation of glycoproteins that translocate to the cell surface from GLUT4-enriched vesicles in an insulin-dependent fashion. *J Biol Chem* 269, 138-142 (1994)

3. K.V. Kandror, L. Coderre, A.V. Pushkin & P.F. Pilch: Comparison of glucose-transporter-containing vesicles from rat fat and muscle tissues: evidence for a unique endosomal compartment. *Biochem J* 307, 383-390 (1995)

4. C.C. Cain, W.S. Trimble & G.E. Lienhard: Members of the VAMP family of synaptic vesicle proteins are components of glucose transporter-containing vesicles from rat adipocytes. *J Biol Chem* 267, 11681-11684 (1992)

5. S.M. Laurie, C.C. Cain, G.E. Lienhard & J.D.Castle: The glucose transporter GluT4 and secretory carrier membrane proteins (SCAMPs) colocalize in rat adipocytes and partially segregate during insulin stimulation. *J Biol Chem* 268, 19110-19117 (1993)

6. K.V. Kandror & P.F. Pilch: Compartmentalization of protein traffic in insulin-sensitive cells. *Am J Physiol* 271, E1-14 (1996)

7. N.J. Morris, A. Ducret, R. Aebersold, S.A. Ross, S.R. Keller & G.E. Lienhard: Membrane amine oxidase cloning and identification as a major protein in the adipocyte plasma membrane. *J Biol Chem* 272, 9388-9392 (1997)

8. N. J. Morris, S.A. Ross, W.S. Lane, C.M. Petersen, S.R. Keller & G.E Lienhard: Sortilin is the major 110 kDa protein in Glut4 vesicles from adipocytes. *J Biol Chem* 273, 3582-3587 (1998)

9. N.J. Morris, S.A. Ross, J.M. Neveu, W.S. Lane & G.E. Lienhard: Cloning and preliminary characterization of a 121 kDa protein with multiple predicted C2 domains. *Biochim Biophys Acta* 1431, 525-530 (1999)

10. N.J. Morris, S.A. Ross, J.M. Neveu, W.S. Lane & G.E. Lienhard: Cloning and characterization of a 22 kDa protein from rat adipocytes: a new member of the reticulon family. *Biochim Biophys Acta* 1450, 68-76 (1999)

11. K.V. Kandror, L. Yu & P.F. Pilch: The major protein of GLUT4-containing vesicles, gp160, has aminopeptidase activity. *J Biol Chem* 269, 30777-30780 (1994)

12. S.R. Keller, H.M. Scott, C.C. Mastick, R. Aebersold & G.E. Lienhard: Cloning and characterization of a novel insulin-regulated membrane aminopeptidase from Glut4 vesicles. *J Biol Chem* 270, 23612-23618 (1995)

13. T. Rogi, M. Tsujimoto, H. Nakazato, S. Mizutani & Y Tomoda: Human placental leucine aminopeptidase/oxytocinase. *J Biol Chem* 271, 56-61 (1996)

14. P.G. Laustsen, S. Vang & T. Kristensen: Mutational analysis of the active site of human insulin-regulated aminopeptidase. *Eur J Biochem* 268, 98-104 (2001)

15. S.R. Keller: Insulin-regulated membrane aminopeptidase. In: Handbook of Proteolytic Enzymes. Eds: Barrett, AJ, Rawlings ND, Woessner JF), *Academic Press*, 1011-1013 (1998)

16. S.A. Ross, H.M. Scott, N.J. Morris, W-Y Leung, F. Mao, G.E. Lienhard & S.R. Keller: Characterization of the insulin-regulated membrane aminopeptidase in 3T3-L1 adipocytes. *J Biol Chem* 271, 3328-3332 (1996)

17. S. Sumitani, T. Ramlal, R. Somwar, S.R. Keller & A. Klip: Expression, localization and insulin regulation of the membrane aminopeptidase vp165 in rat skeletal muscle cells. *Endocrinology* 138, 1029-1034 (1997)

18. D. Malide, J.-F. St-Denis, S.R. Keller & S. W. Cushman: vp165 and GLUT4 share similar vesicle pools along their trafficking pathways in rat adipose cells. *FEBS Lett* 409, 461-468 (1997)

19. J.C. Aledo, L. Lavoie, A. Volchuk, S.R. Keller, A. Klip & H.S. Hundal: Identification and characterization of two distinct intracellular GLUT4 pools in rat skeletal muscle: Evidence for an endosomal and an insulin-sensitive GLUT4 compartment. *Biochem J* 325, 727-732 (1997)

20. S. Martin, J.E. Rice, G.W. Gould, S.R. Keller, J.W. Slot & D.E. James: The glucose transporter GLUT4 and the aminopeptidase vp165 colocalise in tubulo-vesicular elements in adipocytes and cardiomyocytes. *J Cell Science* 110, 2281-2291 (1997)

21. S.A. Ross, J.J. Herbst, S.R. Keller & G.E. Lienhard: Trafficking kinetics of the insulin-regulated membrane aminopeptidase in 3T3-L1 adipocytes. *Biochem Biophys Res Comm* 239, 247-251 (1997).

22. L.A. Garza & M.J. Birnbaum: Insulin-responsive aminopeptidase trafficking in 3T3-L1 adipocytes. *J Biol Chem* 275, 2560-2567 (2000).

23. A. Subtil, M.A. Lampson, S.R. Keller & T.E. McGraw: Characterization of the insulin-regulated endocytic recycling mechanism in 3T3-L1 adipocytes using a novel reporter molecule. *J Biol Chem* 275, 4787-4795 (2000)

24. G.D. Holman & S.W. Cushman: Subcellular localization and trafficking of the GLUT4 glucose

transporter isoform in insulin-responsive cells. *Bioassays* 16, 753-759 (1994)

25. S.A. Ross, S.R. Keller & G.E. Lienhard: Increased intracellular sequestration of the insulin-regulated aminopeptidase upon differentiation of 3T3-L1 cells. *Biochem J* 330, 1003-1008 (1998)

26. J. Yang, A.E. Clark, I.J. Kozka, S.W. Cushman & G.D. Holman: Development of an intracellular pool of glucose transporters in 3T3-L1 cells. *J Biol Chem* 267, 10393-10399 (1992)

27. J.W. Slot, H.J. Geuze, S. Gigengack, G.E.Lienhard & D.E. James: Immuno-localization of the insulin regulatable glucose transporter in brown adipose tissue of the rat. *J Cell Biol* 113, 23-135 (1991)

28. K. Ishii, H. Hayashi, M. Todaka, S. Kamohara, F. Kanai, H. Jinnouchi, L. Wang & Y. Ebina: Possible domains responsible for intracellular targeting and insulin-dependent translocation of glucose transporter type 4. *Biochem J* 309, 813-823 (1995)

29. L.I. Tanner & G. E. Lienhard: Localization of transferrin receptors and insulin-like growth factor II receptors in vesicles from 3T3-L1 adipocytes that contain intracellular glucose transporters. *J Cell Biol* 108, 1537-1545 (1989)

30. J.R. Wu-Wong, C.E. Berg, J. Wang, W.J. Chiou & B.J. Fissel: Endothelin stimulates glucose uptake and GLUT4 translocation via activation of endothelin ETA receptor in 3T3-L1 adipocytes. *J Biol Chem* 274, 8103-10 (1999)

31. J.R. Wu-Wong, C.E. Berg & D. Kramer: Endothelin stimulates glucose uptake via activation of endothelin-A receptor in neonatal rat cardiomyocytes. *J Cardiovasc Pharmacol* 36 (5 Suppl 1), S179-83 (2000)

32. E. Tomas, L. Sevilla, M. Palacin & A. Zorzano: The insulin-sensitive GLUT4 storage compartment is a postendocytic and heterogeneous population recruited by acute exercise. *Biochem Biophys Res Commun* 284, 490-495 (2001)

33. M. Yu, E. Blomstrand, A.V. Chibalin, H. Wallberg-Henriksson, J.R. Zierath & A. Krook: Exercise-associated differences in an array of proteins involved in signal transduction and glucose transport. *J Appl Physiol* 90, 29-34 (2001)

34. A. Zeigerer, M.A. Lampson, O. Karylowski, D.D. Sabatini, M. Adesnik, M. Ren & T.E. McGraw: GLUT4 retention in adipocytes requires two intracellular insulin-regulated transport steps. *Mol Biol Cell* 13, 2421-35 (2002)

35. T. Nagasaka, S. Nomura, M. Okamura, M. Tsujimoto, H. Nakazato, Y. Oiso, N. Nakashima & S. Mizutani: Immunohistochemical localization of placental leucine aminopeptidase/oxytocinase in normal human placental, fetal and adult tissues. *Reprod Fertil Dev* 9, 747-53 (1997)

36. H. Matsumoto, T. Nagasaka, A. Hattori, T. Rogi, N. Tsuruoka, S. Mizutani & M. Tsujimoto: Expression of placental leucine aminopeptidase/oxytocinase in neuronal cells and its action on neuronal peptides. *Eur J Biochem* 268, 3259-66 (2001)

37. M. Nomura, S. Tsukahara, H. Ando, Y. Katsumata, M. Okada, A. Itakura, S. Nomura, F. Kikkawa, T. Nagasaka & S. Mizutani: Differential distribution of placental leucine aminopeptidase/oxytocinase and aminopeptidase a in human trophoblasts of normal placenta and complete hydatidiform mole. *Placenta* 23, 631-9 (2002)

38. S. Toda, H. Ando, T. Nagasaka, S. Tsukahara, M. Nomura, Y. Kotani, S. Nomura, F. Kikkawa, M. Tsujimoto & S. Mizutani: J Existence of placental leucine aminopeptidase/oxytocinase/insulin-regulated membrane aminopeptidase in human endometrial epithelial cells. *Clin Endocrinol Metab* 87, 1384-9 (2002)

39. A.O. Johnson, A. Subtil, R. Petrush, K. Kobylarz, S.R. Keller & T.E. McGraw: Identification of an insulin-responsive, slow endocytic recycling mechanism in Chinese hamster ovary cells. *J Biol Chem* 273, 17968-17977 (1998)

40. M.A. Lampson, A. Racz, S.W. Cushman & T.E. McGraw: Demonstration of insulin-responsive trafficking of GLUT4 and vpTR in fibroblasts. *J Cell Sci* 113, 4065-76 (2000)

41. A.K. El-Jack, K.V. Kandror & P.F. Pilch: The formation of an insulin-responsive vesicular cargo compartment is an early event in 3T3-L1 adipocyte differentiation. *Mol Biol Cell* 10, 1581-1594 (1999)

42. G. Thoidis & K.V. Kandror: A GLUT4-vesicle marker protein, insulin-responsive aminopeptidase, is localized in a novel vesicular compartment in PC12 cells. *Traffic* 2, 577-587 (2001)

43. H. Nakamura, A. Itakuara, M. Okamura, M. Ito, A. Iwase, Y. Nakanishi, M. Okada, T. Nagasaka & S. Mizutani: Oxytocin stimulates the translocation of oxytocinase of human vascular endothelial cells via activation of oxytocin receptors. *Endocrinology* 141, 4481-4485 (2000)

44. D.R. Melvin, B.J. Marsh, A.R. Walmsley, D.E. James & G.W. Gould: Analysis of amino and carboxy terminal GLUT-4 targeting motifs in 3T3-L1 adipocytes using an endosomal ablation technique. *Biochemistry* 38, 1456-1462 (1999)

45. A.O. Johnson, M.A. Lampson & T.E. McGraw: A Di-leucine sequence and a cluster of acidic amino acids are required for dynamic retention in the endosomal recycling compartment of fibroblasts *Mol Biol Cell* 12, 367-381 (2001)

46. J. Ryu, J.S. Hah, J.S. Park, W. Lee, A.L. Rampal & C.Y. Jung: Protein kinase C-zeta phosphorylates insulin-

responsive aminopeptidase in vitro at Ser-80 and Ser-91. *Arch Biochem Biophys* 403, 71-82 (2002)

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

48. A.M. Shewan, B.J. Marsh, D.R. Melvin, S. Martin, G.W. Gould & D.E. James: The cytosolic C-terminus of the glucose transporter GLUT4 contains an acidic cluster endosomal targeting motif distal to the dileucine signal. *Biochem J* 350 Pt 1, 99-107 (2000)

49. J.C. Lawrence Jr, J.F. Hiken & D. E. James: 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 (1990)

50. B.J. Marsh, S. Martin, D.R. Melvin, L.B. Martin, R.A. Alm, G.W. Gould & D.E. James: Mutational analysis of the carboxy-terminal phosphorylation site of GLUT-4 in 3T3-L1 adipocytes. *Am J Physiol* 275(3 Pt 1), E412-22 (1998)

51. S.B. Waters, M. D'Auria, S.S. Martin, C. Nguyen, L.M. Kozma & K.L. Luskey: The amino terminus of insulin-responsive aminopeptidase causes Glut4 translocation in 3T3-L1 adipocytes. *J Biol Chem* 272, 23323-23327 (1997)

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

53. N.W. Chi & H.F: Lodish. Tankyrase is a Golgi-associated mitogen-activated protein kinase substrate that interacts with IRAP in GLUT4 vesicles. *J Biol Chem* 275, 38437-38444 (2000)

54. J.I. Sbodio, H.F. Lodish & N.W. Chi: Tankyrase-2 oligomerizes with tankyrase-1 and binds to both TRF1 (telomere-repeat-binding factor 1) and IRAP (insulin-responsive aminopeptidase). *Biochem J* 361(Pt 3), 451-459 (2002)

55. H. Katagiri, T. Asano, T. Yamada, T. Aoyama, Y. Fukushima, M. Kikuchi, T. Kodama & Y. Oka: Acyl-coenzyme A dehydrogenases are localized on GLUT4-containing vesicles via association with insulin-regulated aminopeptidase in a manner dependent on its dileucine motif. *Mol Endocrinol* 16, 1049-1059 (2002)

56. Y. Shi, S.J. Samuel, W. Lee, C. Yu, W. Zhang, M. Lachaal & C.Y. Jung: Cloning of an L-3-hydroxyacyl-CoA dehydrogenase that interacts with the GLUT4 C-terminus. *Arch Biochem Biophys* 363, 323-32 (1999)

57. P.R. Shepherd & B.B. Kahn: Glucose transporters and insulin action. *NEJM* 341: 248-257 (1999)

58. P.E. Ward, I.F. Benter, L. Dick & S. Wilk: Metabolism of vasoactive peptides by plasma and purified renal aminopeptidase *M Biochem Pharmacol* 40, 1725-1732 (1990)

59. S. Ahmad & P.E. Ward. Role of aminopeptidase activity in the regulation of the pressor activity of circulating angiotensins. *J Pharmacol Exp Ther* 252, 643-650 (1990)

60. G. O'Cuinn, B. O'Connor & M. Elmore: Degradation of thyrotropin-releasing hormone and luteinising hormone-releasing hormone by enzymes of the brain tissue. *J Neurochem* 54, 1-13 (1990)

61. K. Lalu, S. Lampelo & T. Vanha-Perttula: Characterization of three aminopeptidases purified from maternal serum. *Biochim Biophys Acta* 873, 190-197 (1986)

62. M. Tsujimoto, S. Mizutani, H. Adachi, M. Kimura, H. Nakazato & Y. Tomada: Identification of human placental leucine aminopeptidase as oxytocinase. *Arch Biochem Biophys* 292, 388-392 (1992)

63. J.J. Herbst, S.A. Ross, H.M. Scott, S.A. Bobin, N.J. Morris, G.E. Lienhard & S.R. Keller: Insulin stimulates cell surface aminopeptidase activity toward vasopressin in adipocytes. *Am J Physiol* 272, E600-E606 (1997)

64. S. Mizutani, K. Goto, M. Tsujimoto, H. Nakazato, K. Matsuzawa, Y. Furuhashi, K. Arii & Y. Tomoda: Possible effects of placental leucine aminopeptidase on the regulation of brain-gut hormones in the fetoplacental unit. *Biol Neonate* 69, 307-17 (1996)

65. I. Moeller, A.M. Allen, S.Y. Chai, J. Zhuo & F.A. Mendelsohn: Bioactive angiotensin peptides. *J Human Hypertension* 12, 289-93 (1998)

66. E. Mohr, W. Meyerhof & D. Richter: Vasopressin and oxytocin: molecular biology and evolution of the peptide hormones and their receptors. *Vitamins & Hormones* 51, 235-66 (1995)

67. D.R. Corfield, S.E. Webber, Z. Hanafi & J.G. Widdicombe: The actions of bradykinin and lys-bradykinin on tracheal blood flow and smooth muscle in anaesthetized sheep. *Pulm Pharmacol* 4, 85-90 (1991)

68. P.E. Ward, I.F. Benter, L. Dick & S. Wilk. Metabolism of vasoactive peptides by plasma and purified renal aminopeptidase *M Biochem Pharmacol* 40, 1725-1732 (1990)

69. A.L. Albiston, S.G. McDowall, D. Matsacos, P. Sim, E. Clune, T. Mustafa, J. Lee, F.A. Mendelsohn, R.J. Simpson, L.M. Connolly & S.Y. Chai. Evidence that the angiotensin IV (AT(4)) receptor is the enzyme insulin-regulated aminopeptidase. *J Biol Chem* 276, 48623-48626 (2001)

70. J. Kenny: Cell surface peptidases are neither peptide- nor organ-specific. *Trends Biol Sci* 11, 40-42 (1986)

71. S.R. Keller, A.C. Davis & K.B. Clairmont. Mice deficient in the insulin-regulated membrane aminopeptidase show substantial decreases in glucose transporter GLUT4 levels but maintain normal glucose homeostasis. *J Biol Chem* 277, 17677-86 (2002)

72. H.G. Joost, G.I. Bell, J.D. Best, M.J. Birnbaum, M.J. Charron, Y.T. Chen, H. Doege, D.E. James, H.F. Lodish, K.H. Moley, J.F. Moley, M. Mueckler, S. Rogers, A. Schurmann, S. Seino & B. Thorens: Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. *Am J Physiol Endocrinol Metab* 282, E974-976 (2002)

73. H. Jiang, J. Li, E.B. Katz & M.J. Charron: GLUT4 ablation in mice results in redistribution of IRAP to the plasma membrane. *Biochem Biophys Res Comm* 284, 519-525 (2001)

74. L. Maianu, S.R. Keller & W.T. Garvey: Adipocytes exhibit abnormal subcellular distribution and translocation of vesicles containing glucose transporter 4 and insulin-regulated aminopeptidase in type 2 diabetes mellitus: implications regarding defects in vesicle trafficking. *J Clin Endocrinol Metab* 86, 5450-6 (2001)

75. W.T. Garvey, L. Maianu, J.H. Zhu, G. Brechtel-Hook, P. Wallace & A.D. Baron: Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. *J Clin Inves*. 101, 2377-86 (1998)

76. N. Henry: Ginsberg Insulin resistance and cardiovascular disease. *J Clin Invest* 106, 453-458 (2000)

Key Words: Insulin-regulated aminopeptidase IRAP, Placental leucine aminopeptidase P-LAP, Glucose transporter GLUT4, Review

Send Correspondence to: Susanna R. Keller, M.D., University of Virginia, Department of Internal Medicine-Division of Endocrinology, PO Box 801409, Charlottesville VA 22908, USA, Tel: 434-243-5780, Fax: 434-243-9143, E-mail: srk4b@virginia.edu