

Current Topics

Aminopeptidases in Health and Disease

Role of the Insulin-Regulated Aminopeptidase IRAP in Insulin Action and Diabetes

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The insulin-regulated aminopeptidase (IRAP) is a member of the family of zinc-dependent membrane aminopeptidases. It is the homolog of the human placental leucine aminopeptidase (P-LAP). IRAP is expressed in different cell types but has been best characterized in two major insulin target cells, muscle and fat. In these cells IRAP localizes to intracellular membrane compartments under basal conditions. In response to insulin IRAP redistributes to the cell surface. IRAP shares this behavior with the insulin-responsive glucose transporter GLUT4. It is established that insulin's dramatic effect on glucose disposal is mediated through its action on GLUT4. The role IRAP plays in insulin action is unknown. IRAP cleaves several peptide hormones *in vitro*. In insulin-treated cells, concomitant with the appearance of IRAP at the cell surface, aminopeptidase activity toward extracellular substrates increases. Thus, insulin, by bringing IRAP to the cell surface, could increase the processing of extracellular peptide hormones and thereby change their activities. Investigations are underway to determine the *in vivo* substrates for IRAP and to measure the effect of insulin on the cleavage of identified substrates. In individuals with type 2 diabetes the insulin-stimulated translocation of IRAP to the cell surface of muscle and fat cells is impaired. This defect may lead to decreased cleavage and consequently increased action of peptide hormones that are substrates for IRAP. Impaired IRAP action may thus play a role in the development of complications in type 2 diabetes. The findings of decreased expression of GLUT4 and increased heart size in mice in which IRAP was deleted support this hypothesis.

Key words insulin-regulated aminopeptidase; placental leucine aminopeptidase; intracellular trafficking; peptide hormone cleavage; insulin action; diabetes

1. CHARACTERIZATION OF IRAP

The insulin-regulated aminopeptidase was identified as a major protein in intracellular vesicles that were derived from the low density microsomal fractions of adipocytes and muscles.^{1–3} The same vesicles also harbor the insulin-responsive glucose transporter GLUT4 and have been extensively characterized as GLUT4 vesicles.⁴ At the time of its discovery IRAP was named vp165 for vesicle protein of 165 kDa¹ and gp160 for glycoprotein of 160 kDa.²

The purification and cloning of IRAP revealed that it is a type II membrane protein with an N-terminal 109 amino acid cytoplasmic tail, a single transmembrane segment, and a large 894 amino acid extracellular/intralumenal domain⁵ (Fig. 1). The theoretical Mr of IRAP is 117317. However, IRAP is heavily glycosylated and the molecular mass of the processed protein is larger, 165 kDa in most tissues and 140 kDa in the brain.⁵ The central portion of the extracellular/intralumenal domain of IRAP shares homology with the catalytic domains of aminopeptidases A and N and the thyrotropin-releasing hormone degrading enzyme.⁵ It contains the sites that are essential for catalytic function including the zinc-binding site (Fig. 1).^{5,6} These features define IRAP as a member of the family of zinc-dependent aminopeptidases. With the cloning of the human oxytocinase/placental leucine aminopeptidase (P-LAP) it became evident that IRAP and P-LAP were homologues of the same protein.⁷ They are 87% identical at the amino acid level. Most recently IRAP was identified as a receptor for angiotensin IV.⁸

IRAP has a wide distribution and is found in all the major tissues.^{5,7} However, as shown by immunohistochemical

analysis, the expression of IRAP is restricted to specific cell types in the various tissues (9, reviewed in 10). A remarkable characteristic of IRAP is its distinct intracellular localization and its redistribution to the cell surface in response to different stimuli (reviewed in 10).

2. SUBCELLULAR LOCALIZATION OF IRAP AND REGULATION BY INSULIN

The subcellular distribution of IRAP and its regulation by insulin have been best characterized in two major insulin target cells, muscle and fat. IRAP is well expressed in skeletal muscle, heart and white and brown adipose tissues.⁵ In non-stimulated fat cells more than 90% of IRAP localizes to small intracellular vesicles and the endosomal recycling compartment and less than 10% of IRAP is found at the plasma membrane.^{11,12} This distribution of IRAP is dramatically altered by insulin. Within 5 minutes after stimulation of cells with insulin 50% of IRAP is found at the cell surface and the remainder within the intracellular compartments.^{11,13} The absolute amount of IRAP at the cell surface is eight times higher in insulin-stimulated than in non-stimulated fat cells.^{11,14} The characteristic subcellular distribution of IRAP under basal and insulin-stimulated conditions is maintained through a dynamic process that involves the continuous movement of IRAP between the different compartments.^{11,12,15} The differential distribution of IRAP in basal and insulin-stimulated cells is achieved through dramatic changes in the rates of exocytosis of IRAP.^{12,16}

In muscle and fat cells the relative subcellular distribution of IRAP under basal and insulin-stimulated conditions is the

same as for the insulin-responsive glucose transporter GLUT4.^{1–3,14,17} IRAP and GLUT4 are unique among the proteins found in the GLUT4 vesicles. No other GLUT4 vesicle protein exhibits the same efficient intracellular sequestration in non-stimulated cells and marked translocation to the cell surface in response to insulin.

The subcellular distribution of IRAP is distinct from the other known members of the family of zinc-dependent membrane aminopeptidases, aminopeptidases A and N and the thyrotropin-releasing hormone degrading enzyme. These are constitutively expressed at the cell surface.¹⁸ The explanation for this difference lies in the distinct structure of the N-terminal cytoplasmic domain of IRAP.⁵ We have been able to show that the cytoplasmic tail of IRAP carries all the information for its specific intracellular localization and insulin-regulated trafficking.¹⁶ Within the cytoplasmic domain a 29 amino acid sequence (residues 56–84) (Fig. 1) that includes the dileucine motif at position 76/77 preceded by a cluster of acidic amino acids is necessary and sufficient for the dynamic retention of IRAP in the endosomal recycling compartment.¹⁹ The molecular mechanisms that are responsible for the intracellular sequestration and insulin-stimulated translocation of IRAP to the cell surface are under investigation. A few proteins have been identified to interact with the cytoplasmic domain of IRAP (reviewed in 10). Their roles in the intracellular trafficking of IRAP still need to be elucidated.

3. ROLE OF IRAP IN INSULIN ACTION

It is well established that the regulation of the subcellular distribution of GLUT4 in skeletal muscle and fat cells is crucial to the control of glucose homeostasis.²⁰ Insulin, by increasing the number of GLUT4 at the cell surface of muscle and fat cells, increases glucose uptake maximally and thus achieves the disposal of 80–90% of glucose after a meal. The question is what the physiological function of IRAP is and what role it plays in insulin action.

The members of the family of zinc-dependent membrane aminopeptidases, aminopeptidases A and N and the thyrotropin-releasing hormone degrading enzyme, have been shown to process regulatory peptides, thereby changing their activities.¹⁸ Recently, the physiological roles of aminopeptidases A and N in the cleavage and regulation of the actions of angiotensin II and angiotensin III, respectively, have been established.²¹ These precedents suggested that a physiological function for IRAP may also be to process one or several regulatory peptides and modify their activities.

IRAP cleaves several peptide hormones, vasopressin, oxytocin, lys-bradykinin, angiotensins III and IV, and the neuropeptides met-enkephalin and dynorphin A, *in vitro*.^{22–24} Other peptide hormones, among these, insulin, have been tested for cleavage by IRAP and found to be unlikely substrates.²³ Angiotensins III and IV and met-enkephalin are also efficiently cleaved by aminopeptidase N *in vitro*.²⁵ However, IRAP is the only membrane aminopeptidase known to cleave vasopressin and oxytocin, hormones with an N-terminal cystine, *in vitro*. Recent experiments indeed suggest that vasopressin is a specific physiological substrate for IRAP (manuscript in preparation).

A possible role for IRAP in insulin action could thus be

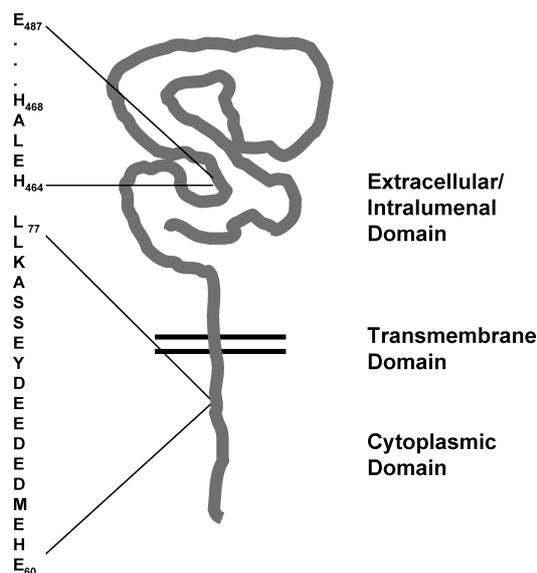


Fig. 1. Domain Structure of IRAP

Highlighted is the 18 amino acid sequence in the cytoplasmic tail that contains the dileucine sequence (aa 76/77) preceded by a cluster of acidic amino acids (aa 60–71). In the extracellular/intralumenal domain the zinc-binding motif (aa 464–468 and aa 487) that is characteristic for the zinc-dependent membrane aminopeptidases is shown.

envisioned as following (Fig. 2). Under basal conditions IRAP is efficiently sequestered within the cells and access to extracellular substrates is limited. Insulin through the activation of its receptors elicits the translocation of IRAP to the cell surface. IRAP thus gains access to its extracellular substrates and efficiently removes the N-terminal amino acids from suitable substrates. In the case of vasopressin, N-terminal cleavage by IRAP initiates its degradation²⁶ and consequently vasopressin action is terminated. Insulin thus, through IRAP, could promote the inactivation of vasopressin and thus control vasopressin action. In support of this model we have found that the cleavage of vasopressin by isolated adipocytes is increased 3-fold by insulin concomitant with the increase of IRAP at the cell surface.²³

To address the question of the function of IRAP in a physiological context we have generated mice with a targeted disruption of the IRAP gene (referred to as IRAP $-/-$ mice). One of the most important findings in the initial characterization of the 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.²⁷ 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 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 is that changes in the action of peptide hormones that are substrates for IRAP lead to a decrease in the synthesis or an increase in the degradation of GLUT4.

The initial characterization of the IRAP $-/-$ mice revealed another interesting finding, an increase in heart size.²⁷ In all the mouse models with decreased or lack of GLUT4 expression cardiac hypertrophies have been observed (re-

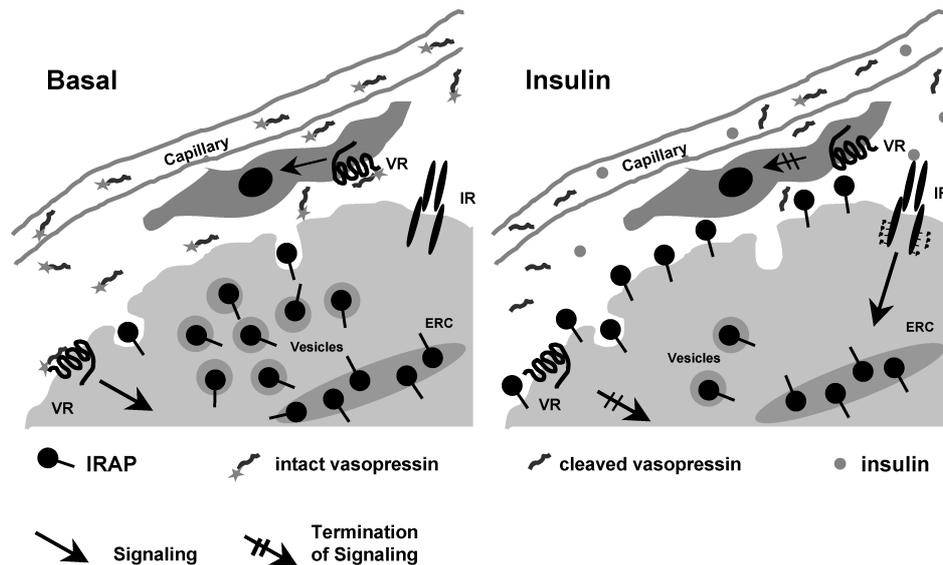


Fig. 2. Differential Subcellular Distribution of IRAP in Basal and Insulin-Stimulated Cells and Regulation of the Cleavage of Extracellular Peptide Hormones

ERC=Endosomal Recycling Compartment, IR=insulin receptor, VR=vasopressin receptor.

viewed in 27). 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 that is a substrate for IRAP remains to be determined. It is noteworthy that several of the peptide hormones found to be substrates for IRAP *in vitro* are vasoactive peptide hormones.²³ If one of these is an *in vivo* substrate for IRAP and is not processed properly in the IRAP $-/-$ mice cardiovascular abnormalities may develop.

4. ROLE OF IRAP IN DIABETES

In individuals with type 2 diabetes insulin action is impaired. One of the consequences of this insulin resistance is a decreased translocation of GLUT4 to the cell surface in response to insulin in muscle and fat cells.²⁸ In addition, the expression of GLUT4 is decreased in fat cells of type 2 diabetics.²⁸ These abnormalities result in diminished glucose disposal and hyperglycemia. Hyperglycemia contributes to the development of the many severe complications found in patients with type 2 diabetes.²⁹ Thus, the impaired function of GLUT4 at the surface of insulin-resistant muscle and fat cells has major detrimental consequences on whole body function.

The levels of IRAP in muscle and fat cells are normal in type 2 diabetics.^{30,31} However, the recruitment of IRAP to the plasma membrane is impaired in skeletal muscle and fat cells from these patients.^{30,31} Consequently it would be expected that IRAP action at the cell surface is diminished in these individuals. Whether the defect in insulin-stimulated IRAP translocation in muscle and fat cells of individuals with type 2 diabetes leads to a decrease in the cleavage and consequently altered action of peptide hormones that are substrates for IRAP needs to be established. The changes we observed in the initial characterization of the IRAP $-/-$ mice, decreased expression of GLUT4 and the enlargement of the heart, both common findings in patients with type 2 diabetes, suggest that impaired function of IRAP at the cell surface

may play a role in the development of complications in insulin-resistant individuals.

The identification of the physiological substrates for IRAP followed by further analysis of the regulation of their cleavage and action by IRAP are required to gain a clear understanding of the physiological function of IRAP and the role it plays in insulin action. This in turn will help to elucidate whether impaired IRAP function may contribute to complications of type 2 diabetes.

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