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Biosynthesis and Regulation of the Insulin Receptor

PHILLIP GORDEN, M.D.,[a] RICHARD ARAKAKI, M.D.,[a]
ELAINE COLLIER, M.D.,[a] AND JEAN-LOUIS CARPENTIER, M.D.[b]

[a]*Diabetes Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland;* [b]*Institute of Histology and Embryology, University of Geneva School of Medicine, Geneva, Switzerland*

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The insulin receptor is an integral glycoprotein of the plasma membrane in most mammalian cells. The gene encodes a 190 kDa proreceptor that undergoes a number of processing steps. The gene is constitutively expressed, but at least one form of regulation has been demonstrated. Glucocorticoids increase the number of insulin receptors on the surface of cultured human lymphocytes, a process which is accompanied by an increase in transcription of the gene. N-linked glycosylation and amide-linked acylation occur as co-translational events. Subsequently, the proreceptor is cleaved into alpha and beta subunits; the subunits then undergo an ester-linked acylation step and N-linked complex glycosylation. In addition, O-linked glycosylation has been recently described in the beta subunit. The mature insulin receptor is inserted into the plasma membrane as an $alpha_2$-$beta_2$ disulfide-linked heterodimer. The receptor can be further regulated on the cell surface by insulin binding and receptor-mediated endocytosis. The receptor concentration on the cell surface then becomes a function of the internalization rate and the receptor recycling rate. Receptor regulation is a relevant feature of many forms of clinical insulin resistance, and recently genetic mutations have been described that determine both the binding properties of the receptor and its translocation and processing properties.

INTRODUCTION

The insulin receptor is a cell surface integral membrane protein present in most mammalian cells. The receptor recognizes and binds insulin; the strength of the insulin signal transduced is a function of the concentration of the insulin receptor complex. Thus, the concentration of insulin, of the receptor, or both, may be varied to regulate the magnitude of the insulin signal.

Early studies employing biochemical techniques defined the insulin receptor as a specific protein and defined its steady state binding parameters. More recent studies employing cell biologic techniques have defined mechanisms of regulation, and molecular genetic studies have elucidated the structure and sequence of the receptor, permitting description of specific functional domains. Clinical studies have utilized all of these techniques to describe the relevance of the insulin receptor to a number of diabetic syndromes.

STRUCTURE OF THE MATURE INSULIN RECEPTOR

The mature receptor that is inserted into the plasma membrane consists of an alpha subunit that contains the insulin-binding domain and a beta subunit that contains a tyrosine kinase domain (Fig. 1). The insulin receptor is composed of a dimer of two

Abbreviation: LDL: low-density lipoprotein

Address reprint requests to: Phillip Gorden, M.D., NIDDK—NIH, Building 31, Room 9A52, Bethesda, MD 20892

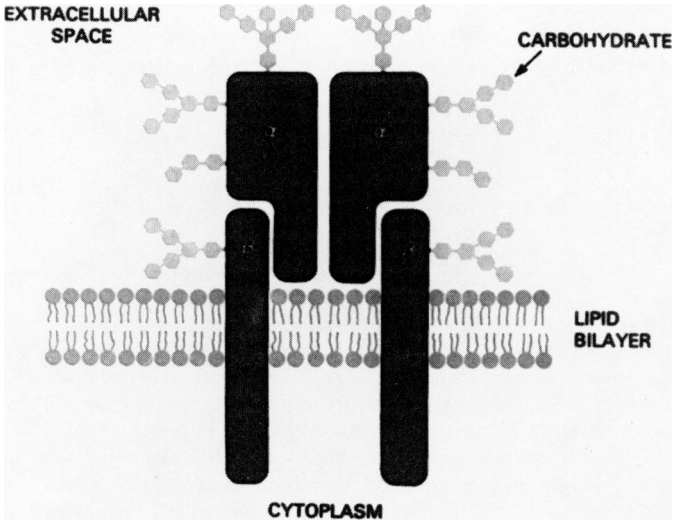

FIG. 1. Structure of the insulin receptor on the cell surface.

alpha and two beta subunits linked by disulfide bonds. Insulin binds to the alpha subunit and activates autophosphorylation of the beta subunit. Once phosphorylated, the beta subunit is an activated tyrosine kinase. The details of other forms of phosphorylation of the insulin receptor and subsequently of its substrates are not well understood. [1]. It seems clear, however, that the binding of insulin and receptor autophosphorylation are necessary, if not sufficient, events to trigger insulin action. It is possible that reagents other than insulin, such as insulinomimetic immunoglobulins, may be able to circumvent part of this pathway [2].

BIOSYNTHESIS OF THE RECEPTOR—THE GENE AND cDNA

The insulin receptor gene is located on chromosome 19, estimated to be greater than 120 kB, and contains 22 exons [3]. Several groups have identified the 5′ flanking region which is noteworthy for the absence of "TATA" and "CAAT" box sequences [3,4,5,6]. There are, however, multiple G-C rich regions that may be possible Spl binding sites, consistent with the promoter sequence for many constitutively expressed "housekeeping" genes. By primer extension analysis, many transcriptional initiation sites have been identified in the 5′ untranslated region. Transcription is induced by glucocorticoids, but no specific glucocorticoid regulatory sequence has been identified [7,8].

The human insulin receptor cDNA (approximately 5 kB) encodes a single chain proreceptor [9,10], which was previously described by biosynthetic labeling studies [11] (Fig. 2). It is interesting that insulin is also synthesized by way of a single chain precursor [12]. The alpha subunit contains a cysteine-rich region, consistent with the ligand-binding domain. There is also a sequence, with four basic amino acids separating the alpha and beta subunits, which is the site of proteolytic cleavage. The beta subunit has been intensely examined by site-directed mutagenesis to determine necessary functional domains. Of interest are the extracellular, transmembrane, tyrosine kinase, and carboxy terminal regions. Superimposed on this protein backbone are a number of processing events.

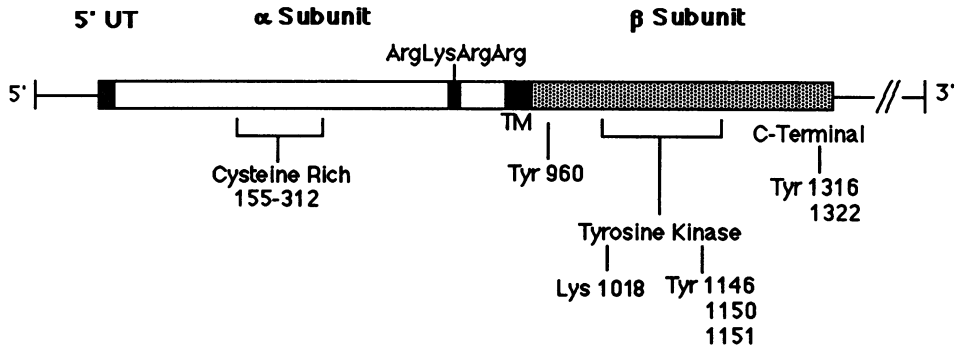

FIG. 2. Schematic representation of the human insulin receptor cDNA. The region of the cDNA designated ArgLysArgArg represents the cleavage site between the α- and β-subunits. The Lys 1018 of the β-subunit is the adenosine triphosphate binding site. The region designated Tyr 1146, 1150, and 1151 represents the biologically significant substrates for receptor phosphorylation. Tyr 960, 1316, and 1322 represent phosphorylation sites of uncertain significance.

For the most part, the insulin receptor gene is transcribed in a constitutive fashion, and the concentration of mRNA is directly proportional to the number of receptors on the cell surface [13]. Aside from developmental regulation, and regulation based on cell transformation, the only known positive regulators of insulin receptor gene expression are glucocorticoids [7,8]. In a time- and dose-related fashion, glucocorti-

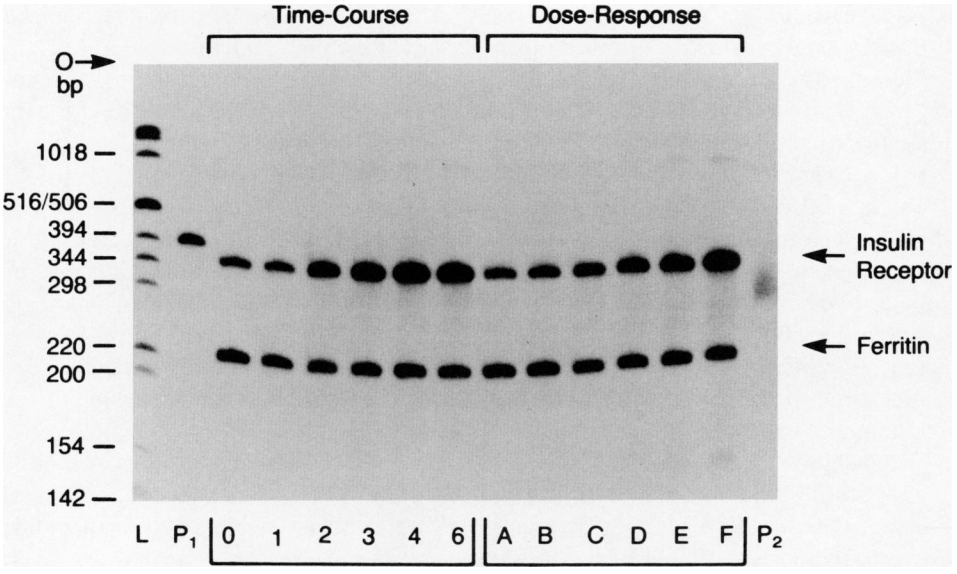

FIG. 3. Effect of hydrocortisone on insulin receptor mRNA. Cytoplasmic mRNA from IM-9 lymphocytes was hybridized to insulin receptor and ferritin anti-sense riboprobes and identified by S_1 endonuclease protection assay. The levels of receptor and ferritin message in cells exposed to increasing concentrations (dose-response A = 0 hydrocortisone, B = 10 nm, C = 50 nm, D = 80 nm, E = 200 nm, and F = 1.4 μm) and longer duration (time course measured in hours) of hydrocortisone are shown. For details of these experiments, see [7].

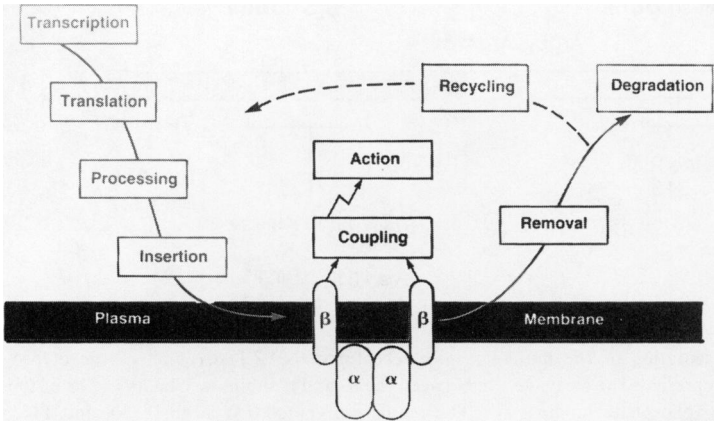

FIG. 4. Regulation of cell surface insulin receptor concentration.

coids increase the number of insulin receptors on the surface of cultured human lymphocytes. This process is related to the synthetic pathway, inasmuch as the receptor turnover rate is not affected. Receptor mRNA levels are proportionally increased, and this increase is a direct transcriptional effect (Fig. 3). While insulin itself will increase proreceptor synthesis to some extent, this increase is not accompanied by an increase in gene transcription [7].

BIOSYNTHESIS—RECEPTOR TRANSLATION

The translation of the insulin receptor message presumably occurs by the same route as other integral membrane proteins, i.e., on membrane-bound ribosomes (Fig. 4). The message encodes a 190 kDa proreceptor protein which undergoes two recognized co-translational events: *(a)* N-linked glycosylation of the high mannose type and *(b)* an amide-linked acylation step. There are 18 potential glycosylation sites on the proreceptor, with 14 on the alpha subunit and four on the extracellular domain of the beta subunit. It is noted that there are 15 potential glycosylation sites on the alpha subunit [9,10], but one of these is composed of asn-pro-ser, which is very unlikely to be glycosylated [14]. As with other proteins, a $Glc_3Man_9GlcNac_2$ oligosaccharide is transferred to an asparagine of the protein via a dolichol phosphate intermediate. This oligosaccharide chain is processed to a complex type of chain by removal of the three glucoses and several mannoses prior to addition of other monosaccharides and sialic acid. If removal of the glucoses is inhibited, the receptor concentration on the cell surface is reduced, suggesting that processing to complex chains is necessary for correct targeting of the receptor to the cell surface [15,16]. Further processing of the high mannose chains to complex chains occurs in the Golgi.

The insulin receptor also contains covalently linked fatty acids. In biosynthetic labeling studies, radiolabeled myristate and palmitate are found attached to the insulin receptor. One of these fatty acids appears to be attached very early, in an amide linkage, as inhibition of protein synthesis prevents its occurrence [17].

As soon as the protein chain is synthesized, possibly as a co-translational event, interchain disulfide dimerization occurs (Fig. 5). Thus, the newly synthesized proreceptor is a disulfide-linked dimer which has undergone high mannose glycosylation and amide-linked acylation. The newly synthesized proreceptor binds insulin but with reduced affinity. The tyrosine kinase activity of the proreceptor is reduced in proportion to the reduced binding affinity (Fig. 6). Thus, the proreceptor, like

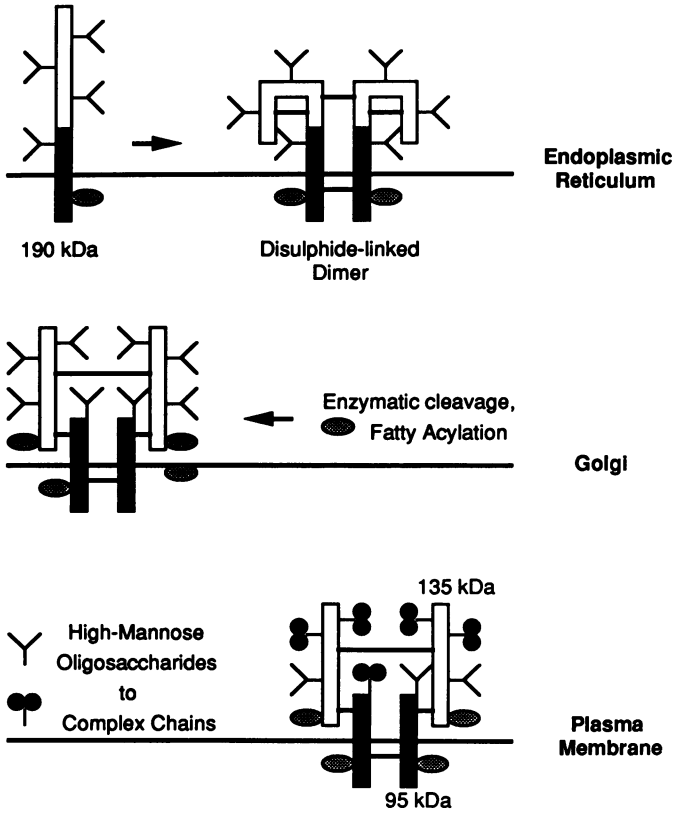

FIG. 5. Scheme of the post-translational processing of the insulin receptor.

proinsulin, is fully active but with diminished affinity as compared to the mature peptide.

PROCESSING OF THE PRORECEPTOR

Further processing of the proreceptor presumably occurs in the Golgi apparatus. Cleavage of the proreceptor to produce the pre-alpha and pre-beta subunits occurs very early, but probably in the Golgi. The mature alpha subunit has an apparent molecular weight of 135 kDa and the mature beta subunit, one of 95 kDa. The insulin receptor contains both high mannose and complex type of N-linked oligosaccharides since the mature receptor contains endoglycosidase-sensitive carbohydrates [18]. The difference in molecular weight of the alpha and beta subunits from that predicted by the amino acid sequence is largely attributed to N-linked glycosylation.

Two additional processing steps less well characterized probably occur in the Golgi. In addition to the amide-linked fatty acid, there is ester linkage of fatty acids to the insulin receptor. Both the alpha and beta subunits contain covalently bound fatty acids [17]. The part that these fatty acids play in receptor function and processing is unknown at the present time.

Recently, another post-translational modification of the insulin receptor has been described, O-linked glycosylation [19]. N-glycanase, an enzyme that specifically removes N-linked oligosaccharides, removes all of the carbohydrate moieties from the proreceptor and the alpha subunit, whereas, in the beta subunit, in addition to

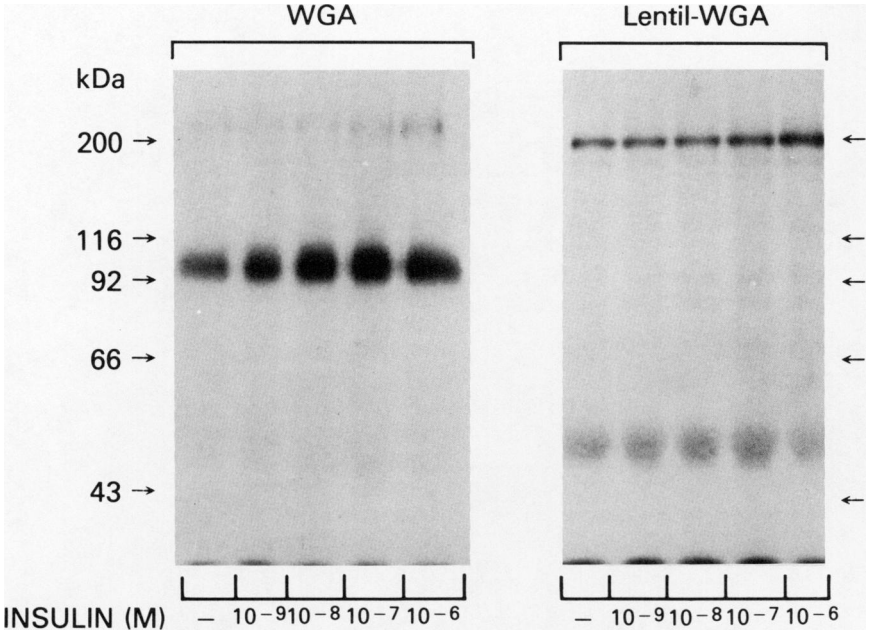

FIG. 6. Insulin dose-response autophosphorylation of immunoprecipitated mature insulin receptor and proreceptor. Mature receptor and precursor from IM-9 cells were isolated by sequential lectin chromatography and immunoprecipitation with site-specific anti-receptor antibody. *Left panel:* Autophosphorylation response to increasing concentrations of insulin in the β-subunit (95 kDa) isolated by wheat germ agglutinin chromatography followed by SDS-polyacrylamide gel electrophoresis and autoradiography. *Right panel:* The proreceptor (190 kDa) isolated by lentil-wheat germ chromatography followed by SDS-polyacrylamide gel electrophoresis and autoradiography. Note the attenuation in phosphorylation in the proreceptor as compared to the mature β-subunit.

N-glycanase, O-glycanase, an enzyme that specifically removes O-linked oligosaccharides, is required to remove all of the carbohydrate. The O-linked carbohydrate is apparently limited to a single tryptic peptide fragment which also contains N-linked carbohydrate. The significance of this newly described O-linked glycosylation to the function or processing of the insulin receptor is presently unknown.

THE FULLY PROCESSED RECEPTOR

Following the processing events described, the mature receptor is transferred from the Golgi to the plasma membrane. The nature of this transfer process is unknown, but it would appear the receptor is inserted into the membrane in a random fashion; however, once inserted into the membrane, the receptor is freely mobile in the plane of the plasma membrane. This finding is based on morphometric studies that have shown that ^{125}I-insulin binds initially in a preferential fashion to the microvillus surface of the membrane and, with time at 37°C, redistributes to the non-villus surface [20]. Other evidence for mobility comes from fluorescent microscopy and from electron microscopic autoradiographic studies that have shown that the insulin receptor complex redistributes to the Golgi pole of the cell when the temperature of initial binding is increased to 37°C. There is no evidence for an extensive intracellular pool of fully processed receptors that can be immediately recruited to the plasma membrane, as reported for the glucose transporter when the cell is stimulated by insulin.

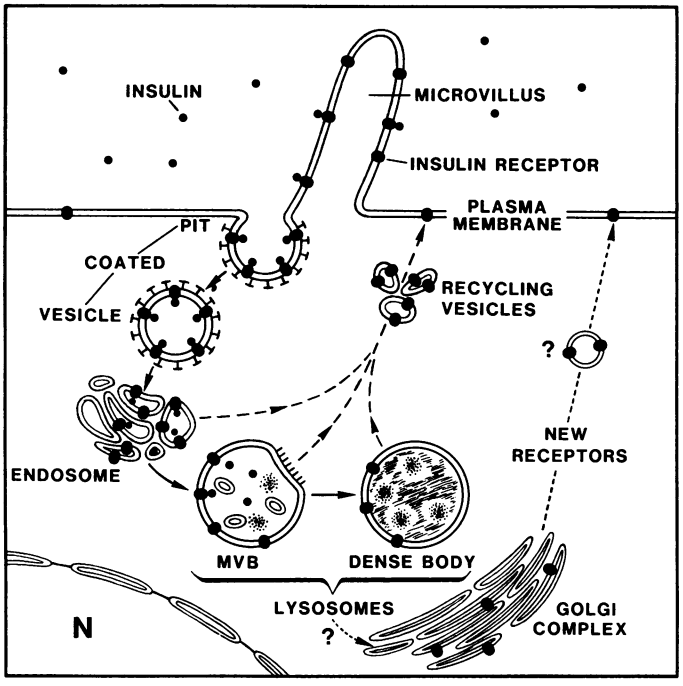

FIG. 7. Scheme of internalization and recycling of the insulin receptor.

The $t^1/_2$ for receptor turnover in cultured human lymphocytes is approximately six to eight hours [21]; this rate is augmented by insulin binding. This "down-regulation" or ligand-induced receptor regulation was the first evidence for a process that appears to be general for most polypeptide hormones [22]. The mechanism of this down-regulation appears to be receptor-mediated endocytosis of the insulin receptor complex [20].

RECEPTOR-MEDIATED ENDOCYTOSIS OF THE INSULIN RECEPTOR COMPLEX

When ^{125}I-insulin, under physiologic conditions, binds to cell surface receptors on cultured or freshly isolated cells, the hormone receptor complex is internalized. Subsequently, a series of intracellular events ensues that can dissociate the hormone from its receptor (Fig. 7).

A number of experimental observations suggest that internalization is the major mechanism by which cell surface insulin receptors are "down-regulated." In cultured human lymphocytes, receptor gene transcription is not affected by down-regulating conditions [7]. In fact, there is a small increase in proreceptor translation under these circumstances [23]. In other cells, there is an effect to inhibit gene transcription analogous to the effect of internalized cholesterol in the down-regulation of the low-density lipoprotein (LDL) receptor [24].

The structures involved in endocytosis appear to be relatively nonspecific and similar for many other polypeptide hormones, growth factors, and other unrelated ligands. Labeled insulin is initially localized in coated pits on the cell surface which invaginates, fuses, and then fissions to form coated vesicles. These vesicles rapidly lose their clatherin coat and progressively become larger non-coated structures called endosomes. The acidification of endosomes promotes dissociation of the ligand receptor

complex, permitting the ligand and receptor to be processed independently [20]. Furthermore, the beta subunit of the receptor with its tyrosine kinase domain oriented toward the cytoplasmic surface could potentially interact with other proteins or structures as an activated tyrosine kinase [25].

The receptor appears to be recycled predominantly from this endosomal compartment based on kinetic analysis, but morphologic data have demonstrated that the receptor can be recycled from other vesicular structures such as lysosomes. Whether there is some interaction of the recycling vesicles with those vesicles involved in the transfer of newly synthesized receptors from the Golgi to the cell surface is at present unknown. Furthermore, it is not clear how specificity is determined, inasmuch as many different polypeptides appear to be recycled in the same or similar vesicles [20]. It is known that certain types of receptors, such as the LDL receptor, recycle continuously, whereas internalization and recycling of the insulin receptor predominantly requires ligand binding. Thus, down-regulation of the receptor is primarily determined by the rate of receptor endocytosis and the rate of receptor recycling.

MECHANISMS REGULATING RECEPTOR-MEDIATED ENDOCYTOSIS

The internalization of the insulin receptor occurs predominantly by way of coated pits [20]. Whether this is an exclusive mechanism, however, is uncertain, inasmuch as exclusive coated-pit internalization has been shown, to date, only for the LDL receptor. Two mechanisms have received recent attention. The first is that insulin receptors with a mutation at the adenosine triphosphate binding site that prevents autophosphorylation are not internalized [26,27], which suggests a possible role for autophosphorylation in the endocytotic event. On the other hand, many ligand receptors such as the growth hormone receptor [28] and the LDL receptor are not phosphorylated. Thus, phosphorylation cannot be a general mechanism regulating endocytosis. The other interesting question is whether the process is driven by a specific amino acid sequence in the intracytoplasmic region of the receptor. In this regard, a consensus sequence among several types of receptors, including the LDL receptor and the class I antigen receptor, has been suggested [29].

RELEVANCE OF THE INSULIN RECEPTOR TO CLINICAL DISEASE

Insulin resistance is a dominant and central feature in obesity, typical type II diabetes, and in several syndromes of extreme alternation of insulin action. Thus, the insulin receptor has a pivotal role in the study of insulin resistance. In obesity and type II diabetes, the receptor is "down-regulated." This ligand-induced regulation, presumably mediated by internalization, decreases the concentration of insulin receptors on the cell surface and, therefore, is a potential factor in clinical insulin resistance; this condition may occur in addition to any other post-receptor defect.

In both type I and type II diabetes, receptor-mediated endocytosis of the insulin receptor is impaired, suggesting that there may be a mechanism for maintaining insulin receptors on the cell surface under circumstances where insulin action is reduced [30,31,32]. Thus "down-regulation" is a feature of most hyperinsulinemic states, whether diabetes is present or not. The impaired internalization appears to be primarily a feature of the hyperglycemic diabetic state and can be reversed with insulin therapy.

Furthermore, several genetic defects have now been elucidated that are associated with extreme forms of insulin resistance (Table 1). In one of these disorders, a specific

TABLE 1
Mutations of the Insulin Receptor Gene Found in Patients with Syndromes of Extreme Insulin Resistance

Mutation[a]		Functional Consequence	References
α-subunit	Phe^{382} to Val	Failure to translocate to the plasma membrane	[33]
Proreceptor	Arg^{723} to Ser	Absence of proreceptor cleavage	[34]
α-subunit[b]	Lys^{460} to Glu	Absence of ligand dissociation with acid pH and temperature	[35]
α-subunit[b]	Glu^{672} to Stop	Premature chain termination	
β-subunit	Gly^{996} to Val	Decrease autophosphorylation	[36]
β-subunit	Trp^{1188} to Ser	Decrease autophosphorylation	[37]

[a]Codon sequence per [10]
[b]Compound heterozygote

mutation in the alpha subunit impairs the translocation of newly synthesized receptors to the plasma membrane, which results in a low concentration of insulin receptors on the plasma membrane and insulin resistance. The second processing defect involves a mutation at the cleavage site which inhibits proreceptor processing to the mature subunits; this defect results in insertion of a low-affinity receptor into the plasma membrane and insulin resistance.

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