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Insulin/IGF-1 Hybrid Receptors: Implications for the Dominant-Negative Phenotype in Syndromes of Insulin Resistance

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Abstract Classical insulin and IGF-1 receptors are $\alpha_2\beta_2$ heterotetrameric complexes synthesized from two identical αβ half-receptor precursors [1,2]. Recent data strongly suggests, however, that nonidentical αβ half-receptor precursors can assemble to generate hybrid holoreceptor species both in vivo and in vitro [3–6,41]. This review focuses primarily on two types of hybrid receptors. The first type is an insulin/IGF-1 hybrid receptor generated by the association of an αβ insulin half-receptor with an αβ IGF-1 half-receptor. The second type is one formed from a wildtype (kinase-active) insulin or IGF-1 αβ half-receptor and a mutant (kinase-inactive) insulin αβ half-receptor. Although the functional properties of insulin/IGF-1 hybrid receptors have not yet been completely defined, wildtype/mutant hybrid receptors are essentially substrate kinase inactive [6]. These data indicate that the mutant αβ half-receptor exerts a transdominant inhibition upon the wildtype αβ half-receptor within the $\alpha_2\beta_2$ holoreceptor complex. This defect in substrate kinase activity may contribute to the molecular defect underlying some syndromes of severe insulin resistance and diabetes. Heterozygous individuals expressing both wildtype and mutant tyrosine kinase-defective insulin receptor precursors demonstrate varying degrees of insulin resistance and diabetes [7–11]. In addition, cell lines which express both endogenous wildtype and transfected kinase-defective insulin receptors display markedly decreased insulin and IGF-1 sensitivity and responsiveness [12–14]. Formation of hybrid receptors which results in premature termination of insulin signal transduction may be one mechanism underlying the observation that kinase-inactive receptors inhibit the function of native receptors.

Key words: insulin/IGF-1 hybrid receptors, autophosphorylation, substrate phosphorylation, protein tyrosine kinase, in vitro assembly

Insulin initiates a variety of cellular responses by binding to a cell-surface glycoprotein receptor. The insulin receptor is minimally composed of two α subunits and two β subunits which are disulfide-linked into an $\alpha_2\beta_2$ heterotetrameric complex (Fig. 1) [1,2]. Individuals with specific, rare genetic defects in the insulin receptor are resistant to the biological action of insulin [15]. Clinical manifestations of syndromes of severe insulin resistance, such as the Type A syndrome and leprechaunism, include compensatory hyperinsulinemia, a skin disorder called acanthosis nigricans, hyperandrogenism, failure to thrive and intrauterine growth retardation, or polycystic ovaries, oligomenorrhea, and hirsutism. Insulin resistance may be due to mutations in the insulin receptor gene that result in decreased numbers of receptors on the cell surface or that impair the normal function of the receptor. In most cases, these patients are either homozygotes with one particular mutation in both alleles or are compound heterozygotes with two distinct genetic defects in each allele. In several reported cases of insulin resistant syndromes, however, individuals were apparently simple heterozygotes with only one defective allele [7–11]. The substantial loss of insulin receptor function in these individuals cannot be accounted for by a simple 50% decrease in insulin receptor levels since the amount of expressed wildtype receptors falls within the normal population range [16]. In addition, one patient with severe insulin resistance also displayed impaired IGF-1 receptor binding and action [17], and overexpression of mutant insulin receptors in cultured cell lines inhibits the function of the endogenous wildtype insulin and IGF-1 receptors [12–14]. Thus, some

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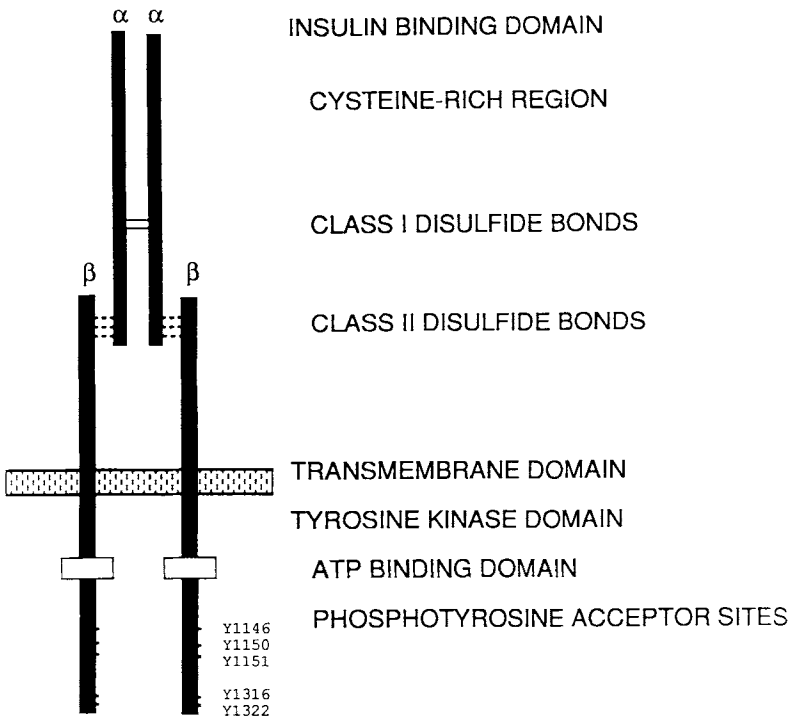

Fig. 1. Schematic representation of an insulin receptor $\alpha_2\beta_2$ heterotetramer. Functional domains are indicated on the right. The α subunits are joined by Class I disulfide bonds to each other (solid lines) and by Class II disulfide bonds to the β subunits (dotted lines). The α subunit contains the insulin binding domain and the cysteine-rich region. The β subunit contains the transmembrane domain (shaded region), the tyrosine kinase domain, the ATP binding sites (open rectangles), and phosphotyrosine acceptor sites (Y1146, Y1150, Y1151, Y1316, Y1322).

genetic defects in the insulin receptor are dominant negative mutations, that is, expression of certain insulin receptor mutant alleles can dramatically interfere with the function of the normal allele gene product [9,12].

Recently, two general models have been proposed to account for dominant-negative insulin receptor mutations (Fig. 2) [18]. Assuming that second messengers of insulin action are endogenous protein substrates that must interact with the insulin receptor, overexpression of a mutant receptor species may compete for these substrates and effectively sequester them from the active, wildtype receptor population. Alternatively, assembly of insulin and IGF-1 receptor subunits into hybrid receptors could generate a functionally inactive hybrid receptor species. Although these hypotheses are not necessarily mutually exclusive, only the hybrid receptor hypothesis has received direct experimental support. This review will focus on recent progress in elucidating the transmembrane signalling properties of hybrid receptors and their possible contribution to insulin resistant states.

INSULIN AND IGF-1 RECEPTOR STRUCTURE AND BIOSYNTHESIS

Insulin and insulin-like growth factor 1 (IGF-1) share extensive structural homology and can initiate a wide variety of biological responses by binding to specific cell-surface glycoprotein receptors on their respective target cells. The insulin and IGF-1 receptors also share similar structural and functional properties [1,2,19]. The insulin receptor binds insulin with high affinity and binds IGF-1 with much lower affinity; conversely, the IGF-1 receptor binds IGF-1 with high affinity and insulin with low affinity. Intensive studies over the past ten years have demonstrated that both receptors are composed of two α subunits (M_r 135,000) and two β subunits (M_r 95,000) covalently linked by disulfide bonds into an $\alpha_2\beta_2$ heterotetrameric complex. The extracellular α subunits are linked to each other through Class I disulfide bonds, a covalent attachment which can be disrupted by DTT reduction in the absence of SDS [20,21]. The α subunits are also anchored to the cell

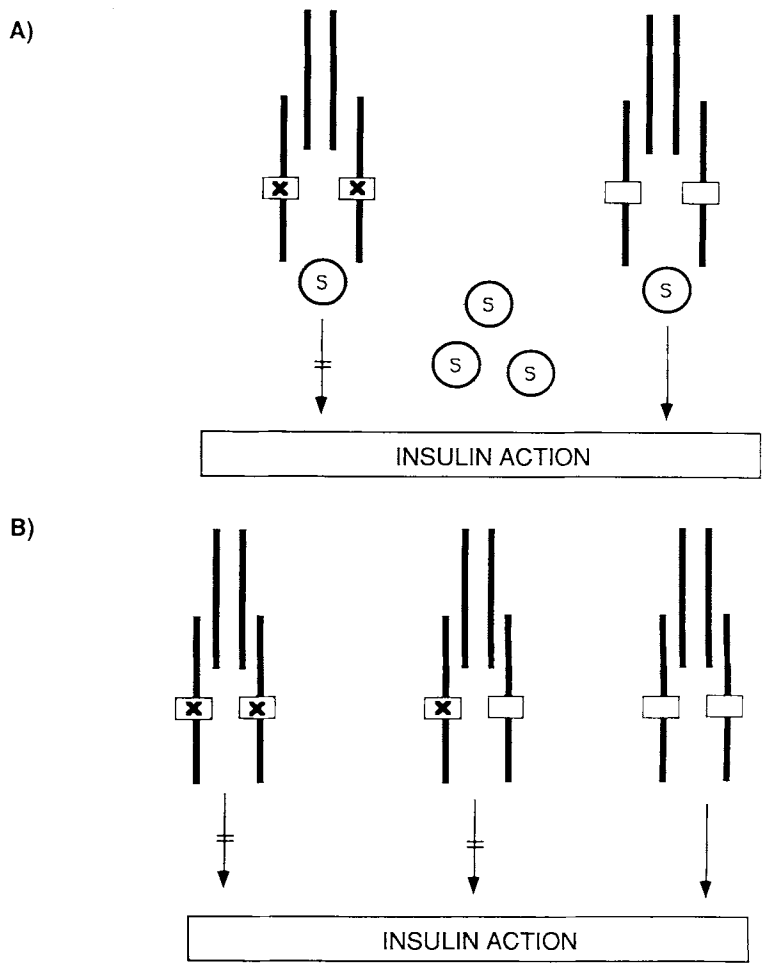

Fig. 2. Molecular mechanisms of negative-dominant insulin receptor mutations. **A:** Substrate competition—wildtype (right) and mutant (left) insulin receptors compete for intracellular substrate (circles labeled with "s"). **B:** Hybrid receptor formation—receptor species present on the cell surface include wildtype receptor (right), mutant receptor (left), and wildtype/mutant hybrid receptor (middle). In both A and B, only the wildtype receptor is capable of transducing the insulin signal. See text for details.

membrane by Class II disulfide linkages to the transmembrane β subunits. These covalent bonds are disrupted by DTT in the presence of SDS. The α subunits contain a cysteine-rich domain which contributes to the high affinity ligand binding site displayed by both insulin and IGF-1 receptors, although other determinants of binding specificity have been identified [22–25]. The transmembrane β subunits contain the intracellular tyrosine-specific protein kinase domain, ATP binding sites, and several phosphotyrosine acceptor sites.

Upon binding insulin, the heterotetramer is activated, and the receptor undergoes autophosphorylation at several tyrosine residues on the β subunit. The major β subunit autophosphorylation sites of the insulin receptor have been identified. Using the nomenclature of Ullrich et al. [26], autophosphorylation occurs primarily on tyrosine residues 1146, 1150, 1151, 1316, and 1322 [27,28]. Due to the extensive sequence identity in this domain, with the exception of the absence of Tyr 1322, equivalent residues in the IGF-1 receptor presumably are the major autophosphorylation sites although this has not been experimentally documented. In any event, phosphorylation of the 1146–1151 region in the insulin receptor activates substrate kinase activity such that ligand occupancy is no longer required [29–31]. The return to an insulin-dependent state requires subsequent β subunit dephosphorylation. The importance of the 1146–1151 region has been demonstrated by both kinetic data and the observation that a mutant

form of the insulin receptor lacking the carboxy terminal 43 amino acids including Tyr 1316 and 1322 has similar protein kinase activity compared to the wildtype receptor [28,32]. The IGF-1 receptor substrate kinase activity is likewise activated by prior autophosphorylation [33].

In addition to sharing similar structural and functional properties, similar events occur during the expression of the insulin and IGF-1 receptors. The synthesis and assembly of the insulin receptor can be summarized in four general steps [1,34–38]: 1) a 155-kDa polypeptide pre-proreceptor is synthesized, cotranslationally acylated, and core-glycosylated, resulting in a 190-kDa protein in the endoplasmic reticulum; 2) intramolecular disulfide bonds are formed resulting in an apparent increase in molecular weight to 210 kDa; and the monomeric precursors assemble into a disulfide-linked dimer; 3) the dimer is proteolytically cleaved to generate an $\alpha_2\beta_2$ heterotetramer; and 4) terminal sialic acid residues are added to the carbohydrate chains and the mature receptors are transported to the cell surface. High-affinity ligand binding and tyrosine-kinase activity emerge only after proteolytic cleavage and require the mature $\alpha_2\beta_2$ heterotetrameric state. Since the $\alpha\beta$ half-receptor precursors undergo considerable processing, the possibility arises that nonidentical $\alpha\beta$ half-receptor subunits may be randomly sorted into various $\alpha_2\beta_2$ heterotetrameric complexes, resulting in the formation of hybrid receptors (Fig. 3). This prediction has been supported by experimental evidence as described below. In addition, the assembly of hybrid receptors requires that both receptor precursors be translated simultaneously and does not occur via post-translational rearrangement of the Class I disulfide bonds in the mature cell surface $\alpha_2\beta_2$ heterotetrameric holoreceptors (unpublished data).

EVIDENCE FOR INSULIN/IGF-1 RECEPTOR HYBRIDS

Several recent reports support the existence of naturally occurring insulin/IGF-1 hybrid receptors in human placenta and in several cultured cell lines [3–5]. Soos and Siddle have demonstrated that anti-insulin receptor monoclonal antibodies precipitate IGF-1 binding from solubilized extracts of human placenta and tissue culture cell membranes [4,5]. Treatment of the receptor complexes with alkaline pH/DTT, to selectively reduce the Class I disulfide bonds

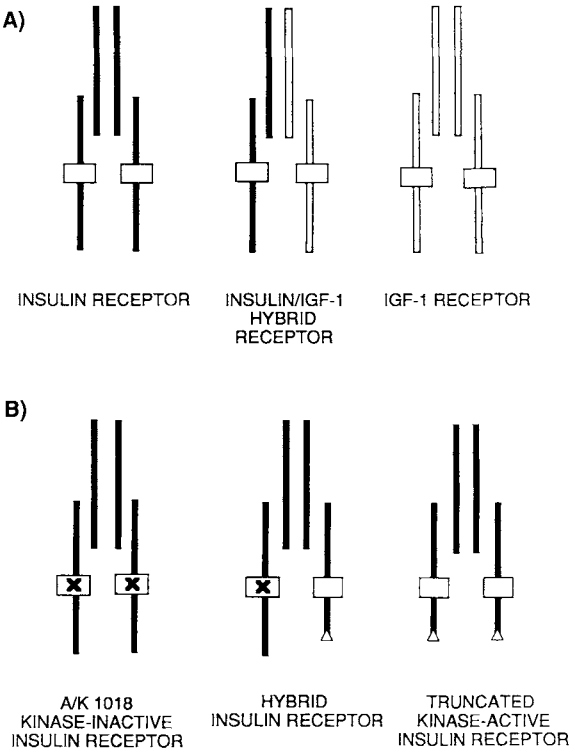

Fig. 3. Schematic representation of two types of hybrid receptors. **A:** Insulin receptor (left), insulin/IGF-1 hybrid receptor (middle), IGF-1 receptor (right). **B:** Mutant (kinase-inactive) insulin receptor (left), mutant/truncated hybrid insulin receptor (middle), truncated (kinase-active) insulin receptor (right).

between α subunits, prevented the same insulin receptor monoclonal antibodies from immunoprecipitating IGF-1 binding. This occurred concomitant with a shift in subunit association state from an $\alpha_2\beta_2$ heterotetramer to an $\alpha\beta$ heterodimer state. The simplest interpretation of these data is that the original $\alpha_2\beta_2$ holoreceptor complex was an insulin/IGF-1 hybrid receptor composed of an insulin $\alpha\beta$ half-receptor and an IGF-1 $\alpha\beta$ half-receptor.

Moxham and colleagues also used immunological criteria to demonstrate hybrid receptors in the HepG2 human liver and NIH3T3 mouse fibroblast cell lines [3]. In these cell lines, size heterogeneity was observed in the IGF-1 β subunit resulting in a doublet of M_r 102,000 and 95,000 when visualized by reducing SDS-polyacrylamide gel electrophoresis. This difference had previously been attributed to differences in post-translational modification [39]; however, after removing the carbohydrate moiety with glycosidases, subtle differences in molecular weight persisted. Several properties of the M_r 95,000 β subunit, including apparent molecular

weight and tryptic phosphopeptide maps, were similar to the insulin receptor β subunit. Moreover, antibodies prepared against a peptide corresponding to the carboxy terminus of the insulin receptor β subunit cross-reacted with this same IGF-1 receptor-derived β subunit [40]. To establish that the M_r 95,000 IGF-1 receptor β subunit was actually an insulin receptor β subunit present in an insulin/IGF-1 hybrid receptor complex, anti-insulin receptor antibodies were used to precipitate these receptor species in the presence or absence of DTT [3]. As expected, following reduction of the Class I disulfide bonds in the heterotetrameric complexes, only the M_r 95,000 β subunit was precipitated with the anti-insulin receptor antibody whereas in the absence of reduction both the M_r 95,000 and 102,000 β subunits were immunoprecipitated. Taken together, these data strongly indicate that insulin/IGF-1 hybrid receptors naturally occur in both human placenta and tissue culture cell lines. Whether or not these hybrid receptors are also present in typical insulin target tissues in vivo such as adipose and skeletal muscle remains to be determined.

FUNCTIONAL PROPERTIES OF HYBRID RECEPTORS

It is well documented that insulin and IGF-1 have distinct but overlapping biological actions. Assigning specific effects to insulin or IGF-1 in a particular cell type has been difficult due to the ability of each ligand to bind, at least weakly, to both receptor species. In addition, some of the biological responses mediated by both hormones may result from activation of insulin/IGF-1 hybrid receptors. Defining the functional properties of these native hybrid receptor species in terms of ligand binding specificity, protein kinase activity and intracellular signalling is important to our understanding of insulin and IGF-1 action. To date, such characterization is incomplete, although reportedly the insulin/IGF-1 hybrid receptor binds IGF-1 with higher affinity than insulin, and IGF-1 is more potent in activating hybrid receptor autophosphorylation [3,5,40]. These data suggest that insulin/IGF-1 hybrid receptors may actually function more like IGF-1 receptors than insulin receptors, although the effects on down-stream signalling have not yet been evaluated.

As described previously, expression of defective insulin receptors both in patients and in cultured cell lines is a dominant phenotype resulting in insulin resistance. In cultured fibroblasts, high level expression of a kinse-defective insulin receptor (A/K1018) also completely inhibits IGF-1 stimulated mitogenesis and results in a significant proportion of the endogenous wildtype IGF-1 receptors to be assembled into a mutant insulin/IGF-1 hybrid receptor complex [5,12]. Consequently, the defect in both insulin and IGF-1 action may result from disruption of the normal signalling pathway due to the presence of inactive hybrid holoreceptors composed of one wildtype αβ half-receptor and one mutant αβ half-receptor. To address this issue, we developed a biochemical approach to generate various hybrid holoreceptor species in vitro [6,41]. Assembly of the defective-kinase A/K1018 insulin αβ half-receptor with either the wildtype IGF-1 or insulin αβ half-receptor resulted in the formation of $\alpha_2\beta_2$ holoreceptors which were completely devoid of substrate kinase activity. These data directly demonstrated that the mutant half-receptor suppresses the normal function of the wildtype half-receptor in a mutant/wildtype hybrid receptor complex, consistent with the inhibition of both insulin and IGF-1 action associated with the expression of a kinase-defective insulin receptor in intact cells.

The ability to obtain defined $\alpha_2\beta_2$ hybrid holoreceptor species in vitro also provided a powerful tool to examine the molecular mechanisms involved in ligand-mediated transmembrane activation. Although these mutant/wildtype hybrid receptors were devoid of substrate kinase activity, we were surprised to observe that β subunit autophosphorylation was apparently unaffected. To account for this dissociation between β subunit autophosphorylation and substrate kinase activity, a defect in the receptor intramolecular signalling cascade was proposed. To examine this apparent defect, hybrid receptors composed of a kinase-defective half-receptor assembled with a truncated, kinase-active half-receptor were prepared. Similar to the mutant/wildtype hybrid receptor, this mutant/truncated species was also substrate kinase-inactive. Furthermore, within these hybrid receptors, autophosphorylation of the inactive β subunit was observed, whereas autophosphorylation of the truncated, functional β subunit was blocked. Thus, these data suggest that β subunit autophosphorylation occurs via an intramolecular trans-phosphorylation reaction. Moreover, the absence of substrate kinase activity in mutant/wildtype hybrid receptors as well as in het-

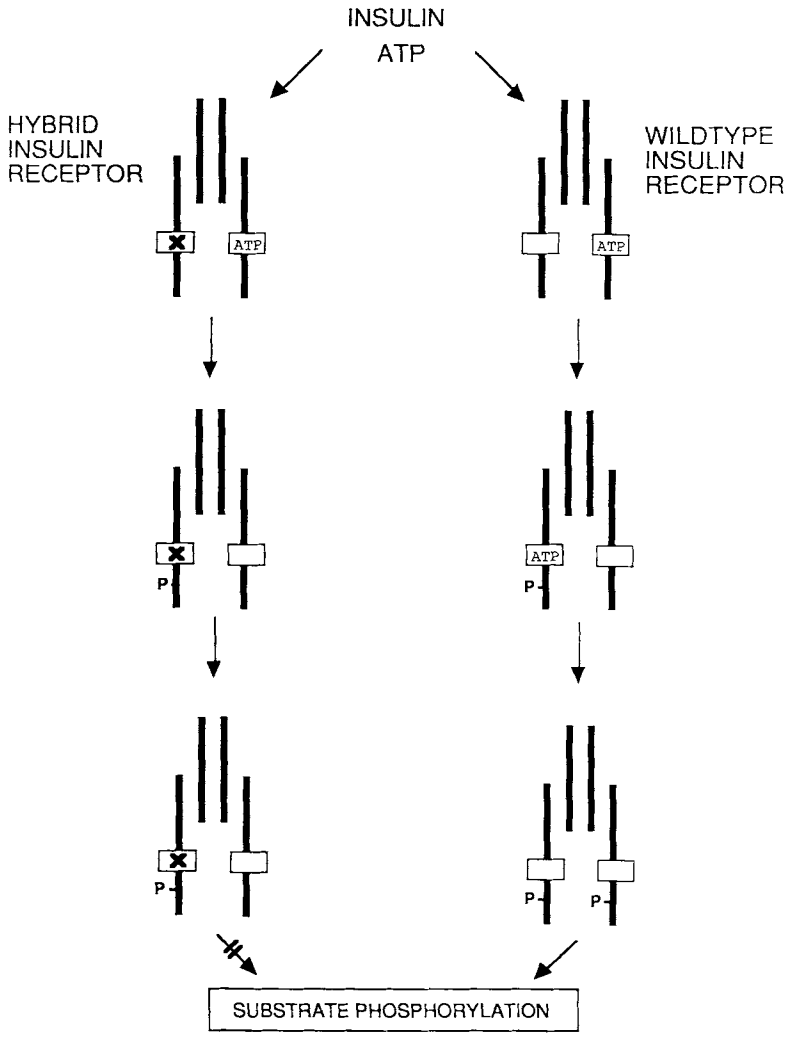

Fig. 4. Postulated intramolecular β subunit autophosphorylation pathway. Mutant/wildtype hybrid insulin receptors (left) autophosphorylate in an intramolecular-trans reaction but are substrate kinase-inactive since only the kinase-defective β subunit is phophorylated; in contrast, wildtype receptors (right) autophosphorylate in an intramolecular-trans reaction followed by additional secondary phosphorylation, and become an activated substrate kinase probably due to phosphorylation of both β subunits.

erotetrameric insulin receptors containing one normal and one proteolytically fragmented β-subunit (42) suggests that two functional β subunits are required for substrate kinase activation. A schematic representation of the intramolecular β subunit autophosphorylation pathway based upon these data is depicted in Figure 4.

PROSPECTS

With the exception of the transmembrane region, naturally occurring mutations in virtually every domain of the insulin receptor have been described which detrimentally affect expression of mature receptors on the cell surface or affect the ability of the receptor to function [15,43]. Mutations impairing kinase activity of the receptor not only have severe consequences in homozygotic individuals, but also result in substantial insulin resistance in heterozygotic patients. A molecular explanation of insulin resistance despite the presence of one normal insulin receptor allele, which also accounts for simultaneous defects in IGF-1 signalling, is the formation of dysfunctional wildtype/mutant hybrid receptors in vivo. Consequently, an important issue is to demonstrate the presence of these hybrid receptors in the heterozygous patient population which display altered receptor function.

Assuming that these types of hybrid receptors are actually present in insulin-resistant patient populations, appropriate model systems must be

developed in order to systematically study the physiological and biochemical properties of both wildtype and mutant hybrid receptors. There are several approaches to this problem, including the use of transfected tissue cultured cells as well as the generation of transgenic animals. Clearly, both systems will be needed to examine the complex interplay between the homologous insulin and IGF-1 receptors and heterologous hybrid receptors.

The natural presence of hybrid receptors will also provide a useful biochemical tool to dissect the molecular events involved in ligand-stimulated transmembrane signalling. For example, the ability to isolate hybrid receptors with dissimilar ligand-binding domains presents an opportunity to determine the symmetry and stoichiometry of activation of the kinase domain by insulin. In addition, events in receptor biosynthesis may be clarified by studying the formation of hybrid receptors, and the molecular events by which the binding of insulin results in the activation of the tyrosine kinase domain may be delineated in a careful study of the flow of information within hybrid receptor complexes. A detailed understanding of the transmembrane signalling cascade could potentially lead to therapeutic interventions to correct the molecular defect of mutant/wildtype hybrid receptors.

Finally, although many of the previous reports of immunologically cross-reactive insulin and IGF-1 receptors can be accounted for by insulin/IGF-1 hybrid receptors, several properties of the observed insulin-related and IGF-1 receptor types are not consistent with the properties of an insulin/IGF-1 hybrid receptor. For example, several studies have identified receptors with high affinity for both IGF-1 and IGF-2 [44] or high affinity for insulin and IGF-2 [45–48]. In addition, a fetal IGF-1 receptor species which is distinct from both the insulin and adult IGF-1 receptor has been reported [49]. Whether these atypical insulin and IGF receptor species occur from various combinations of alternative splicing, post-translational modification or are the products of distinct genes remains an exciting area of future investigation.

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