Structure of the Receptor for Insulin-Like Growth Factor II: The Puzzle Amplified

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The insulin-like growth factor II (IGF-II) is a polypeptide hormone with structural homologies to insulin and insulin-like growth factor I (IGF-I). In contrast to these other hormones, the in vivo function of IGF-II is not known. Although IGF-II can stimulate a broad range of biological responses in isolated cells, these responses have usually been found to be mediated by the insulin and IGF-I receptors. Recently, the receptor for IGF-II was found to also be the receptor for mannose-6-phosphate. Since this latter receptor has been implicated in targeting of lysosomal enzymes, the question is now raised of whether the same protein can also mediate metabolic responses to IGF-II.

INSULIN-LIKE GROWTH FACTOR II (IGF-II) IS A POLYPEPTIDE hormone whose physiological function has been a long-standing puzzle in endocrinology (*1*). In contrast, two structurally related hormones, insulin and insulin-like growth factor I (IGF-I), have been implicated in numerous responses. In vivo, insulin is one of the primary regulators of rapid anabolic responses, including glucose uptake into muscle and fat cells, glycogen synthesis in liver, and fat synthesis in adipocytes (*2*). The importance of insulin in regulating these processes is demonstrated in the condition known as diabetes mellitus, in which there are defects in the synthesis of insulin or the ability of cells to respond to insulin (*3*). The administration of insulin to these individuals can reverse the symptoms of this condition.

IGF-I appears to be one of the primary regulators of the growth of an organism (*1, 4*). It is mainly produced in the liver in response to growth hormone. Although growth hormone may have some direct effects on cells, its ability to stimulate growth appears to be mediated primarily by way of IGF-I (*5*) because (i) infusion of IGF-I into growth hormone–deficient rats can restore growth (*6*), (ii) injections of IGF-I directly into the tibial epiphyseal plate can stimulate cartilage proliferation (*7*), and (iii) certain pygmies in Africa can produce normal amounts of growth hormone but have decreased concentrations of serum IGF-I (*8*).

The physiological role of IGF-II is not known. Growth hormone deficiency causes a partial decrease in the plasma concentrations of IGF-II (*1*). However, in pygmies with decreased IGF-I, the plasma concentration of IGF-II is near normal (*8*). Thus, IGF-II alone is not sufficient to stimulate growth. Also, nude mice with IGF-II–producing tumors and, consequently, elevated IGF-II do not show an increased growth rate (*9*). In rats, plasma levels of IGF-II decrease dramatically after birth, suggesting that IGF-II may play a role in fetal development (*10*). However, in humans, plasma levels of IGF-II actually increase after birth, supporting the view that, in humans, IGF-II's role is not limited to fetal development (*11*). The messenger RNA (mRNA) for IGF-II is present in many cells in the body (*12*). IGF-II mRNA is also elevated in several tumor cells, suggesting that IGF-II could act as a paracrine or autocrine growth factor (*13*). However, the processing of IGF-II mRNA is complex (*14, 15*). Much of the IGF-II mRNA is of a large size that cannot be used in translations in vitro to produce IGF-II hormone (*15*). Moreover, certain cells with high levels of IGF-II mRNA do not produce the IGF-II protein (*16, 17*). This could mean that the IGF-II mRNA codes for a protein other than the IGF-II hormone (*15*).

These three polypeptide hormones each have their own distinct receptors (*1*). The receptors for insulin and IGF-I share a number of properties. Each is composed of two distinct polypeptides of M_r ~130,000 and 90,000. The two receptors also can bind each other's ligand, although with a ~100 times weaker affinity than the homologous ligand. Both receptors also have intrinsic tyrosine-specific kinase activities that, by biochemical and immunological criteria, are closely related (*18*). The tyrosine kinase activity of the insulin receptor, and presumably of the IGF-I receptor, appears to be required for insulin to stimulate all of its biological responses in cells (*2*). The isolation of the complimentary DNAs (cDNAs) that encode these two receptors led to the demonstration that these receptors also share sequence homology (*19*). The amino acid sequences of the kinase domains of the two receptors are most homologous, being 85% identical.

The putative IGF-II receptor is quite different from the insulin and IGF-I receptors. (In this discussion I use the term "IGF-II receptor" although, as it will become clear later, it has not been proved that this molecule propagates a signal after binding IGF-II.) The IGF-II receptor is composed of a single polypeptide of M_r ~250,000 (*20*) and has no intrinsic kinase activity (*21*). Antibodies to the insulin and the IGF-I receptors do not cross-react with the IGF-II receptor (*1*). Although the IGF-II receptor binds IGF-II with high affinity (a dissociation constant of $\simeq$1 n*M*) it does not bind either insulin or IGF-I (*22*). IGF-II itself, however, can bind to the insulin and IGF-I receptors. The affinity of IGF-II for these two receptors varies considerably in different reports (*1*). It is not clear if these differences are due to the particular preparations of ligands used or to heterogeneity in the insulin and IGF-I receptors.

The presence of all three different receptors in most cells has complicated the assignment of a particular response to a particular receptor. In general, the insulin receptor has been implicated in more rapid anabolic responses, whereas the IGF-I receptor is more likely to mediate proliferative responses of cells (*23*). However, insulin stimulates a proliferative response in certain cells through its own receptor (*24*), and the IGF-I receptor can in other cells mediate

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rapid anabolic responses (*25*). In a recent study, a rapid intravenous injection of IGF-I into humans had the same glucose lowering ability as insulin (*26*).

Attempts to determine which responses are mediated through the IGF-II receptor have been less successful. Some investigators have assigned particular responses to the IGF-II receptor on the basis of correlations between binding studies and activity curves (*27*). However, several studies in which specific antibodies were used to block responses through the three different receptors led to the conclusion that the IGF-II responses examined were mediated through either the IGF-I or insulin receptor (*28, 29*). It has even been proposed that the IGF-II receptor does not play a role in transmembrane signaling (*28*).

In the last year, however, evidence from several systems has suggested that IGF-II can stimulate a response through its own receptor. The responses so linked include stimulation of a calcium ion influx in 3T3 fibroblasts (*30*), amino acid uptake in human myoblasts (*31*), DNA synthesis in a human erythroleukemia cell line and a rat cell line (*32*), and glycogen synthesis in hepatoma cells (*33*). In two studies, the responses to IGF-II were not completely blocked by specific antibodies to the insulin and IGF-I receptors (*31, 33*). In one study, an antibody to the IGF-II receptor was even found to stimulate a particular biological response, the activation of glycogen synthesis (*33*).

The recent isolation and sequencing of the IGF-II receptor cDNA has forced a reevaluation of these data (*34*). The amino acid sequence of the IGF-II receptor predicts a structure with only a single transmembrane region, an extracellular domain comprising 93% of the receptor molecule, and a relatively small cytoplasmic domain of M_r 18,000. Neither the extracellular nor the intracellular domains of the IGF-II receptor share homology with the insulin or IGF-I receptor. Thus, one might think that such a receptor would be unlikely to mediate responses similar to those of the insulin and IGF-I receptors. However, the receptor for nerve growth factor also has a small cytoplasmic domain with no apparent signal-transducing abilities (*35*), yet this receptor can mediate some of the same responses as the insulin receptor (*36*). Such receptors must be coupled to other proteins for signaling. For example, a recent study suggests that the IGF-II receptor is linked to a pertussis toxin–sensitive G protein (*37*).

Identity of IGF-II Receptor with Receptor for Mannose-6-Phosphate

The most surprising finding is the 80% identity between the sequence of the human IGF-II receptor (*34*) and that of the bovine cation-independent mannose-6-phosphate (Man-6-P) receptor (*38*). It has been proposed that the Man-6-P receptor participates in the delivery of lysosomal enzymes to the lysosome (*39*). Since the two cDNAs were isolated from different species (human and cow for the IGF-II and Man-6-P receptors, respectively), this degree of sequence identity is consistent with a single gene encoding both proteins. Additional support for this hypothesis came from the finding that mRNA synthesized from the isolated cDNA could be used by frog oocytes to produce a protein that binds IGF-II and is recognized by antibodies to the Man-6-P receptor (*34*). More recently, purified human (*40*) and rat (*41*) IGF-II receptors have been found to interact with antibodies to the Man-6-P receptor and with Man-6-P. Also, the purified Man-6-P receptor was found to bind IGF-II with the same high affinity as IGF-II binds to its own receptor (dissociation constant, K_d, of 0.2 nM) and a stoichiometry of one IGF-II molecule per molecule of Man-6-P receptor (*42*). Finally, the amino acid sequence of the human Man-6-P receptor was found to be 99.4% identical with the sequence of the human–IGF-II receptor (*43*).

These results raise the question of whether one receptor can mediate two such dissimilar physiological roles as the metabolic responses to IGF-II and the lysosomal targeting of proteins. For example, it is possible that the binding of IGF-II to the Man-6-P receptor has no physiological function. If so, there could be another IGF-II receptor present on cells whose presence would be masked by the rather large amounts of the Man-6-P receptor. This hypothetical IGF-II receptor could be in the same family as the insulin and IGF-I receptors (that is, have an intrinsic tyrosine kinase activity) and be responsible for mediating the metabolic responses to IGF-II. The presence of such a receptor could explain some of the anomalous binding data that have been reported (*44*) and the difficulty in demonstrating biological responses through the Man-6-P receptor (*28, 29*).

Alternatively, the IGF-II/Man-6-P receptor might mediate the cellular responses to IGF-II and not participate in lysosomal delivery of proteins. The role of the phosphomannosyl moiety in targeting various acid hydrolases to the lysosomes was first indicated by studies of cells from patients with I-cell disease (*39*). The fibroblasts of these patients were found to lack the phosphotransferase activity required to synthesize the phosphomannosyl recognition marker. Since these fibroblasts also released some of their newly synthesized lysosomal enzymes into the medium, it was hypothesized that a receptor for the phosphomannosyl moiety normally binds these proteins in the Golgi complex and directs them to the lysosome. A protein that binds phosphomannosyl residues of apparent M_r 215,000 was subsequently purified from liver (*45*). A variety of studies support a role for this cation-independent Man-6-P receptor in the targeting of lysosomal enzyme. First, the intracellular localization and movements of this protein are consistent with this role (*39*). Second, antibodies to this Man-6-P receptor have been found to disrupt lysosomal delivery of proteins (*46*). Third, cells deficient in the Man-6-P receptor show increased secretion of lysosomal enzymes (*47*). Finally, recent studies of this expressed Man-6-P receptor in cells transfected with its cDNA have shown that it can target endocytosed β-glucuronidase to lysosomes (*43*).

However, other studies indicate alternative pathways for lysosomal targeting. Another receptor for Man-6-P (called the cation-dependent form) has been described (*48*), and this protein also appears to participate in lysosomal targeting of proteins (*39*). Systems other than those recognizing the Man-6-P moiety must also play a role in directing lysosomal enzymes to their proper compartment since the fibroblasts from the patients with I-cell disease have normal lysosomal levels of such enzymes as β-glucocerebrosidase and acid phosphatase. In addition, other cell types from these same patients have nearly normal levels of lysosomal enzymes, although these cells are also deficient in their phosphotransferase activity (*39*). Receptors that bind Man-6-P but do not play a role in lysosomal targeting have also been identified. For example, a receptor (called the homing receptor) that may be responsible for lymphocyte attachment to the endothelial venules of lymphoid organs also binds the phosphomannosyl moiety (*49*).

Although it is unusual for one protein to have two such dissimilar functions, there is precedence for a single protein binding and responding to two distinct ligands. One such example is the receptor for the neurotransmitter acetylcholine. This receptor also binds thymopoietin (a hormone that regulates thymocyte differentiation) with high affinity and thymopoietin affects neuromuscular transmission (*50*). Also, a single bacterial protein (the *tar* protein) can mediate additive and independent responses to two distinct ligands (aspartate and maltose) (*51*). Thus, the IGF-II/Man-6-P receptor may participate in both processes. Indeed, it is possible that the

presence of both the Man-6-P and IGF-II binding activities in the same protein may allow an integration of two distinct signals. For example, the IGF-II/Man-6-P receptor might respond to membrane or circulating proteins containing Man-6-P as well as circulating IGF-II. The ability of Man-6-P to increase the affinity of its receptor for IGF-II (*40, 41*) would allow these two signals to act synergistically. Such a network of growth-regulating receptors interacting with carbohydrates and growth factors has been suggested (*52*).

The identification of the IGF-II receptor as the receptor for Man-6-P also suggests a mechanism by which insulin may inhibit protein degradation in cells (*53*). Since insulin induces a translocation of the IGF-II/Man-6-P receptor from an intracellular site to the plasma membrane (*54*), this translocation might decrease the amount of lysosomal enzymes present in cells by disrupting the movement of newly synthesized enzymes from the Golgi to the lysosomes. Such an effect could explain insulin's ability to inhibit intracellular protein catabolism. This mechanism of insulin action would be analogous to insulin's effect on glucose uptake, a process that is mediated via insulin's inducing a translocation of glucose transporters from an intracellular site to the plasma membrane (*55*). This proposed mechanism would also explain why inhibitors of intracellular trafficking selectively inhibit insulin's ability to decrease intracellular proteolysis (*56*).

Thus, the finding that the IGF-II and Man-6-P receptors are related, if not identical, proteins suggests new avenues of investigation. It also further compounds the puzzle of the physiological role of IGF-II and its receptor. However, with the recent production of recombinant IGF-II (*57*) and the availability of a cDNA clone for the IGF-II receptor (*34*), the pace of research in this area should greatly accelerate.

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