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Growth, Differentiation, and Survival: Multiple Physiological Functions for Insulin-Like Growth Factors

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Stewart, Claire E. H., and Peter Rotwein. Growth, Differentiation, and Survival: Multiple Physiological Functions for Insulin-Like Growth Factors. *Physiol. Rev.* 76: 1005–1026, 1996.—The insulin-like growth factors (IGFs), IGF-I and IGF-II, comprise a conserved pair of secreted proteins with diverse effects on growth, development, and metabolism. Insulin-like growth factor action is initiated upon binding to cell-surface receptors and is modulated through interactions with secreted IGF binding proteins (IGFBPs). The last decade has seen an explosion of new information about the physiological roles of the IGFs. In this review, we critically examine this information from biochemical, cell biological, and molecular genetic perspectives. We discuss the structures and functions of the two IGF receptors, outline the actions of the six IGFBPs, and summarize and interpret recent studies highlighting essential roles for components of the IGF system in the growth and development of the embryo and fetus, in tissue differentiation, in cell survival and proliferation, and in cancer. These results are discussed in the context of new opportunities for understanding the mechanisms of IGF action in multiple biological processes.

I. INTRODUCTION

The importance of peptide growth factors in growth and development is well established. Like other multifunctional growth factors, insulin-like growth factors I and II (IGF-I and IGF-II) elicit diverse effects on a variety of biological processes in cell culture systems and have a broad range of functions in the embryo, fetus, and adult. The last decade has seen an explosion of information about the IGFs. Complementary DNAs and genes encoding both growth factors have been cloned and characterized from vertebrate species as diverse as fish and humans; the structure of the two IGF receptors has been established, and insights into their modes of action described; and a family of IGF binding proteins has been discovered and characterized. This review attempts to summarize and interpret this new information in the context of the functions of the two IGF receptors and the roles of IGF-I and IGF-II in cellular and whole animal physiology. As the title indicates, emphasis is placed both on "traditional" IGF actions on growth and on newer observations relating to the roles of the IGFs in development, cell survival, and tissue differentiation. Because of the vast scope of the IGF field, this review focuses on papers published since 1990. Earlier studies are discussed only to provide appropriate historical context. Readers are directed to several recent reviews on different aspects of the IGF field for additional information (9, 40, 84, 95, 212).

II. HISTORICAL OVERVIEW

A. Insulin-Like Growth Factors I and II

The IGF field originated in three disparate areas of biomedical research: investigation into the actions of growth hormone (GH) on growth, studies on the insulin-like effects of components of serum, and assessment of the role of locally secreted factors on cell replication. In 1957, Salmon and Daughaday (178) found that the effects of GH on cartilage growth in the rat occurred through a serum factor, initially designated sulfation factor because their assay monitored incorporation of radiolabeled sulfate into extracellular matrix. This factor, which was subsequently termed somatomedin C (48), ultimately was shown to be IGF-I (171). In the 1960s, several laboratories sought to identify components in serum with insulin-like effects on metabolism that were not neutralized by anti-insulin antibodies (76, 132). These substances became known as nonsuppressible insulin-like activity and ultimately were shown to include both IGF-I and IGF-II. In the early 1970s, Pierson and Temin (156) observed that rat liver cells in tissue culture secreted their own "multiplication-stimulating activity" that could enhance cell replication. This factor was eventually identified as IGF-II (172).

Initial attempts to purify these disparate factors suggested that they had overlapping activities. As a consequence, the term *insulin-like growth factor* was proposed in 1976 (170) to signify the relationship of these substances to insulin and to emphasize their growth-promoting activities. With the purification and sequencing of human IGF-I and IGF-II in in the late 1970s, it was found that IGF-I was a single-chain basic protein of 70 amino acids and that IGF-II was a slightly acidic single-chain peptide of 67 residues (171, 172). Insulin-like growth factors I and II are ~70% identical to one another, and their A and B domains are ~50% identical to the A and B chains of human insulin, indicating the appropriateness of this nomenclature. The advent of molecular cloning in the 1980s has proven that both IGFs are highly conserved proteins found in an array of vertebrate species (for recent reviews, see Refs. 63, 176).

B. Insulin-Like Growth Factor Receptors

With the availability of highly purified IGFs in the mid 1970s, researchers began to perform cell surface binding assays in an attempt to determine whether these ligands interacted with the insulin receptor (126). It was soon shown that iodinated IGF-I bound to a receptor that was distinct from the insulin receptor (133). In 1980, a third receptor was identified that preferentially bound IGF-II (165). The subsequent purification of each receptor and cloning of their cDNAs demonstrated that each was the distinct product of a unique gene (124, 138, 209).

C. Insulin-Like Growth Factor Binding Proteins

Unlike insulin, circulating IGFs are bound to carrier proteins, now known as IGF binding proteins (IGFBPs). The first indications of the existence of IGFBPs came in the mid 1970s, when it was shown that radioiodinated IGFs complexed with serum proteins, resulting in recovery after neutral chromatography of IGFs in the molecular mass range of 50–150 kDa and not 7.5 kDa (230). It soon was suggested that these serum carrier proteins had at least two functions: prolongation of the half-life of circulating IGFs and neutralization of their metabolic effects (229). Through protein purification and cloning experiments, it is now known that the IGFBP family comprises at least six members, and a diversity of functions has been ascribed to these proteins (9, 17, 95).

III. MEDIATORS OF INSULIN-LIKE GROWTH FACTOR ACTION

A. Insulin-Like Growth Factor I Receptor

1. *Structural considerations*

The IGF-I receptor (IGF-IR) is a heterotetrameric glycoprotein composed of two ligand-binding α-subunits of 706 amino acids and two transmembrane β-subunits of 627 residues (209) (Fig. 1). The human protein is produced by mRNAs derived from the single 21-exon IGF-IR gene, located on chromosome 15q25-q26 (1, 209). The receptor is synthesized as a single preproprotein of 1,367 amino acids. After cotranslational removal of the 30-residue signal peptide, the precursor is glycosylated and cleaved at the Arg-Lys-Arg-Arg tetrapeptide at amino acids 707–710 to generate α- and β-subunits (209). These are linked together by disulfide bonds to form $\alpha\beta$-half receptors, which are joined by disulfide bonds between the α-subunits to form the mature $\alpha_2\beta_2$-receptor (Fig. 1). The IGF-IR is similar in topography and sequence to the insulin receptor and shares >50% amino acid identity (209).

The α-subunit contains the ligand-binding region of the receptor. The IGF-IR binds IGF-I with a dissociation constant (K_d) of <1 nM in intact cells; IGF-II binds with severalfold lower affinity, and insulin with more than 100-fold lower affinity (95, 115). In general, biological potency parallels binding affinity. Domain-swapping experiments with the insulin receptor have implicated a cysteine-rich segment as being necessary for high-affinity binding of IGF-I (5, 83, 182), and antibodies directed to this region

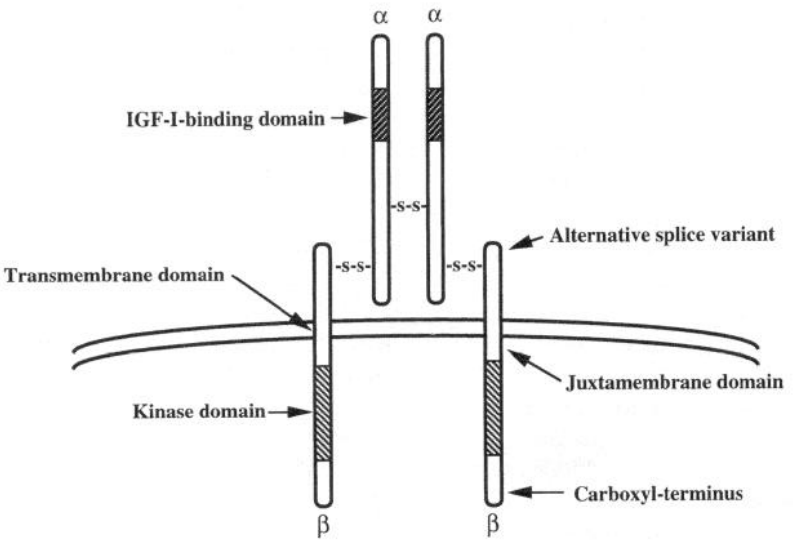

FIG. 1. Insulin-like growth factor (IGF)-I receptor. Functional domains within α- and β-chains are labeled, and disulfide bonds involved in maintaining receptor heterotetramer are indicated.

block IGF-I binding (83). Surprisingly, in the insulin receptor, segments amino- and carboxyl-terminal to the analogous cysteine-rich portion mediate high-affinity binding of insulin (103, 182). The precise binding site for IGF-II on the IGF-IR has not been determined.

The β-subunit is composed of a short extracellular domain, a membrane-spanning segment, and a large intracytoplasmic region containing a tyrosine kinase domain and sites of tyrosine and serine phosphorylation (115, 209) (Fig. 1). Ligand binding to the α-subunit triggers activation of the intracellular tyrosine kinase, possibly by stimulating a conformational change in the β-subunit, leading to receptor autophosphorylation by an intramolecular *trans*-mechanism similar to that used by other receptor tyrosine kinases (74, 99, 115) (see Fig. 2). As described below, tyrosine phosphorylation is an essential activating step for receptor function. The unliganded α-subunit thus functions in the holoreceptor as a repressor of the intracellular tyrosine kinase; repression can by relieved only by IGF binding (115).

Several receptor variants have been described. Variable RNA processing, involving an alternative splice site at the 5′-end of exon 14, leads to synthesis of receptor isoforms with either Thr Gly or Arg in the extracellular part of the β-chain (227). Both types of β-chains are expressed in multiple tissues and cell types (227). In studies in which each isoform was overexpressed in Chinese hamster ovary cells, the variant containing Thr Gly showed less ligand-induced endocytosis and mediated enhanced autophosphorylation and phosphorylation of insulin receptor substrate 1 (IRS-1) (42). It is not known if similar functional differences occur in vivo.

Variation in glycosylation has been found for both the α- and β-chains in several tissues and in cultured cells, including human placenta, the central nervous system, rat fetal skeletal muscle, the mouse C2 myoblast cell line, human neuroblastoma cell lines, and others (reviewed in Ref. 115). The functional significance of these differences has not been determined in most instances. Although it is likely that the variability in subunit mobility seen on sodium dodecyl sulfate-polyacrylamide gel electrophoresis is caused by differences in glycosylation, the existence of additional receptor isoforms with differences in amino acid composition has not been ruled out completely.

Because of the similarity in structure and sequence between the IGF-I and insulin receptors, receptor hybrids can form (31, 115). These chimeras consist of $\alpha\beta$-dimers of the IGF-IR linked to $\alpha\beta$-dimers of the insulin receptor by disulfide bonds (191, 205). Receptor hybrids are found in several tissue and cell types (3, 140, 191) and probably exist in all cells in which both IGF-I and insulin receptors are expressed. The ligand binding properties of hybrids resemble the IGF-IR: high affinity for IGF-I and much lower affinity for insulin (191). Signaling properties appear to be intermediate. Because receptor activation occurs via *trans*-phosphorylation (74), a mutation in the tyrosine kinase domain of either β-chain would impair the function of its mate. Kinase-defective insulin receptors have been described in patients with diabetes mellitus (201); in theory, these mutants, if complexed to form hybrid receptors, also could inhibit IGF-I action.

2. *Signal transduction through the insulin-like growth factor I receptor*

The characterization of IRS-1 as an intracellular docking protein mediating many of the actions of insulin (reviewed in Refs. 143, 221) has facilitated functional analysis of the IGF-IR. Activation of the IGF-IR by ligand binding causes the rapid tyrosine phosphorylation of IRS-1 and the intracytoplasmic assembly of a complex consisting of

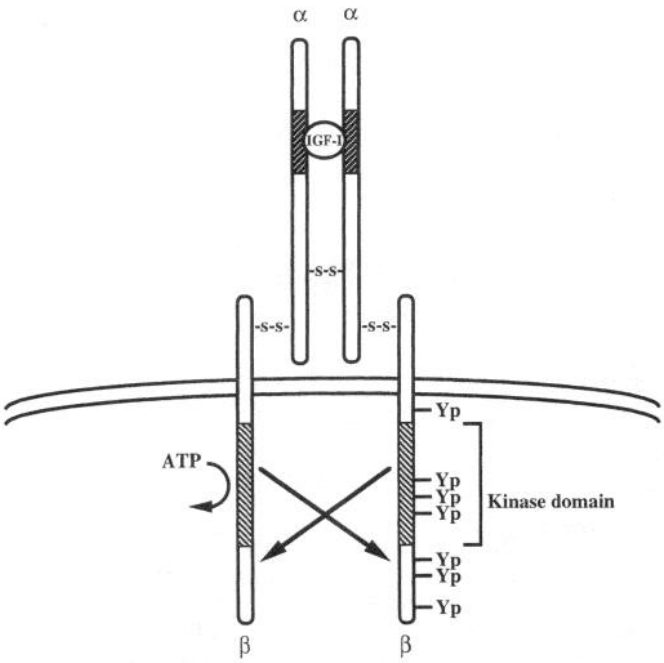

FIG. 2. Signal transduction by IGF-I receptor. Ligand binding to extracellular cysteine-rich region within α-chains induces activation of intracellular tyrosine kinase domain on each β-subunit. Autophosphorylation on tyrosine residues occurs by a transphosphorylation mechanism. Cysteine-rich and kinase domains are depicted by hatched boxes. Phosphorylated tyrosines are indicated. See text for details.

a variety of proteins that are responsible for stimulation of diverse downstream signal transduction pathways (reviewed in Refs. 115, 219) (see Table 1). Binding of IRS-1 to the IGF-IR appears to be mediated by tyrosine phosphorylation of an Asn Pro Glu Tyr motif, located at residues 947–950 of the β-chain (88, 136, 225). An analogous tyrosine at position 960 in the insulin receptor has a similar function. The newly discovered homologue of IRS-1 (195), IRS-2, may engage the insulin receptor and the IGF-IR through similar mechanisms.

Tyrosine phosphorylation of IRS-1 on multiple sites creates a series of docking motifs for intracellular proteins with *src* homology 2 (SH2) domains (115, 142). These SH2 domains comprise a region of ~100 amino acids that share sequence similarity with a segment first identified in the cellular oncogene c-*src* (reviewed in Ref. 38). Different proteins containing SH2 domains bind to distinct docking sites based both on recognition of a phosphorylated tyrosine and on the sequence of amino acids adjacent to this modified tyrosine (142, 221). These proteins include enzymes and additional adaptor molecules, as outlined in Table 1. The binding of the adaptor Grb2 and its associated GDP:GTP exchange protein, Sos, by IRS-1 leads first to the recruitment of these proteins to the cell membrane, and then to interaction with and stimulation of the protooncogene p21 *ras*. Activation of p21 *ras* in turn initiates stimulation of the mitogen-activated protein (MAP) kinase pathway and induction of a variety of biological effects, including regulation of gene expression (reviewed in Ref. 50). Grb2-Sos also can be activated through another adaptor, Shc (*src* homology domain-containing protein; Ref. 154), which can bind directly to the IGF-I and insulin receptors (31, 115), as can several other signaling intermediates (21, 184). Activation of another key enzyme, phosphatidylinositol (PI) 3-kinase, also leads to induction of several biological effects, including stimulation of hormone-sensitive glucose transport (31, 98, 115) and activation of the enzyme p70 S6 kinase, which may be involved in mitogenesis (32).

The importance of IRS-1 in mediating effects of insulin and IGF-I has been demonstrated by studies of IRS-1-deficient mice (7, 199). These animals survive and have normal blood glucose levels but show impaired growth and diminished insulin-stimulated glucose uptake (7, 199). Because IRS-1-deficient mice are not completely refractory to the effects of either hormone, these results underscore the existence of additional signaling pathways activated by each peptide, including those potentially mediated by IRS-2 (195), or regulated by direct interactions of signaling molecules with each receptor (184, 224).

3. *Structure-function relationships*

Manipulations of IGF-I in the whole animal have validated the hypothesis that IGF-I signaling pathways play key roles in somatic growth (51, 117, 129). Similarly, the introduction of modified receptors into cells has begun to provide information on essential domains and critical amino acids involved in different aspects of receptor function. This section reviews recent developments in dissecting structure-function relationships within the intracellular part of the IGF-IR.

Vast overexpression of human IGF-IRs in mouse and rat fibroblasts has been found to cause ligand-dependent

TABLE 1. *SH2-containing proteins that bind to IRS-1 or IGF-IR*

Name	Function	Specificity
Grb2	Adaptor, binds mSos; Grb2-mSos complex activates p21 *ras*	IRS-1, IGF-IR
GAP	*Ras* GTPase-activating protein	IGF-IR
SH-PTP2	Protein tyrosine phosphatase; also called PTP1D or Syp	IRS-1, IGF-IR
p85	Regulatory subunit of PI 3-kinase; p110 is the catalytic subunit	IRS-1, IGF-IR
Nck	Adaptor protein, unknown activity	IRS-1
Shc	Adaptor protein, can bind to the Grb2-mSos complex	IRS-1, IGF-IR (?)

SH2, *src* homology 2; IRS-1, insulin receptor substrate 1; IGF-I, insulin-like growth factor I; IGF-IR, IGF-I receptor; PI, phosphatidylinositol.

neoplastic transformation, as measured by enhanced colony formation in soft agar and the development of tumors when transfected cells were introduced into immunodeficient nude mice (96). These studies highlight a potential role for the IGF-IR in tumorigenesis, a topic that is discussed in section IV*D1*. Similar results have been observed by expressing wild-type IGF-IRs in fibroblasts in a receptor-deficient background (186). Surprisingly, while a transfected IGF-IR can support anchorage-independent growth of fibroblasts lacking endogenous receptors, the insulin receptor cannot (137). Moderate overexpression of IGF-IRs has been shown to lead to increased cellular proliferation, with IGF-I being the only growth factor needed to stimulate transit through the cell cycle (15, 186). Conversely, the absence of IGF-IRs causes a marked reduction in the rate of replication of fibroblasts in culture, with slowing of all phases of the cell cycle (186).

The intracellular kinase domains of the IGF-I and insulin receptors are ~84% identical (209) and activate several of the same signaling intermediates, including IRS-1 and Shc, as noted above (31, 115, 143). Despite the extensive structural homology between these segments, analysis of chimeric receptors in fibroblasts has revealed that the intracytoplasmic portion of the IGF-IR is 10 times more potent in stimulating DNA synthesis after ligand addition than the analogous part of the insulin receptor (109). In contrast, effects on glucose transport showed a comparable dose potency (109). The regions responsible for the more potent growth-promoting properties of the IGF-IR have not been identified, although they are likely to involve segments that show less conservation with the insulin receptor, such as the juxtamembrane and carboxyl-terminal regions (61 and 44% identity, respectively; Ref. 209). In this regard, a chimeric IGF-IR containing the last 112 amino acids of the insulin receptor in place of the final 121 homologous residues showed only slightly increased stimulation of glycogen synthesis, PI 3-kinase activity, and MAP kinase activity in response to IGF-I than the wild-type IGF-IR after transfection into NIH-3T3 fibroblasts. Stimulation of thymidine incorporation into DNA was not altered (200). Similar results were observed when the last 107 amino acids of the insulin receptor were substituted for the final 99 residues of the IGF-IR (69). Thus the mitogenic actions of the IGF-IR do not appear to map to the carboxyl-terminal of the protein.

Analysis of deletion and substitution mutations within the β-chain of the receptor has yielded additional new information about IGF-IR function. As expected, the data demonstrate that receptor tyrosine kinase activity is required for most signaling functions. A mutation of lysine-1003, the ATP binding site, completely disrupts receptor function in transfected NIH-3T3 fibroblasts (99). Minimal tyrosine phosphorylation occurred in response to IGF-I, and ligand binding did not trigger activation of PI 3-kinase, glucose uptake, or DNA synthesis (99). Receptors containing β-chains truncated at amino acid 952, and thus lacking the kinase domain, did not transmit growth-promoting signals and acted as dominant-negative inhibitors when transfected into cells expressing wild-type receptors (159). A similarly nonfunctional receptor was created by substitution of tyrosines-1131, -1135, and -1136 with phenylalanine (81, 100). Alteration of all three tyrosines, which are the major sites of receptor autophosphorylation in the IGF-IR (and analogously in the insulin receptor; Ref. 31), resulted in no autophosphorylation, no phosphorylation of cellular substrates, including IRS-1 and Shc, diminished ligand-activated receptor internalization, and no short-, medium-, or long-term biological effects (81, 100). Mutation of individual residues in this triple tyrosine cluster also caused a decrease in the extent of autophosphorylation and diminished IRS-1 and Shc phosphorylation (86, 116, 192). While substitution of phenylalanine for tyrosine at residues 1131 or 1135 did not inhibit induction of DNA synthesis or cellular proliferation in response to IGF-I in receptor-deficient fibroblasts transfected with each mutant, modification of tyrosine-1136 did reduce replication (116). In contrast, each substitution mutation blocked colony formation in soft agar. Taken together, these results demonstrate that each tyrosine in this cluster is not equivalent and indicate that a fully tyrosine phosphorylated and presumably fully functional receptor is required for anchorage-independent growth but not for mitogenesis.

As noted earlier, tyrosine-950 is essential for binding and phosphorylation of IRS-1 (88, 136, 225), and the analogous amino acid tyrosine-960 plays the same role in the insulin receptor (31). Substitution of phenylalanine for tyrosine at this site did not alter autophosphorylation or ligand-activated receptor internalization, but blunted phosphorylation of IRS-1 and blocked other biological effects (136, 225). This tyrosine also has been found to bind to another signaling intermediate, the *ras* GTPase-activating protein (GAP) (184). More extensive mutations, deleting residues 947–950 or 944–965 of the juxtamembrane domain, additionally abrogated IGF-I-stimulated receptor internalization (88, 225).

Mutation of tyrosine-1316 near the carboxyl-terminal of the IGF-IR also interrupts protein-protein interactions between the activated IGF-IR, the regulatory subunit of PI 3-kinase p85, and the tyrosine phosphatase SH-PTP2 (21, 184). The functional consequences of these mutations have not been elucidated.

Two tyrosines at positions 1250 and 1251 of the IGF-IR are not found in the insulin receptor (209). Substitution of either or both amino acids with phenylalanine had a minimal effect on autophosphorylation and substrate phosphorylation in receptor-deficient fibroblasts transfected with these mutants, and did not alter cellular prolif-

eration in response to IGF-I (137). In contrast, the double mutation or substitution for tyrosine-1251 alone caused a profound decrease in the efficiency of cellular transformation, as measured by diminished colony growth in soft agar (21, 137). These results demonstrate that the mitogenic and transforming properties of the IGF-IR can be dissociated and present an experimental model to test the hypothesis that distinct signal-transduction pathways mediate each biological effect.

Additional determinants within the carboxyl-terminal tail of the IGF-IR may be involved in mediating cellular transformation. After transfection into receptor-deficient fibroblasts, receptors lacking the last 108 amino acids showed ligand-dependent autophosphorylation, mediated phosphorylation of IRS-1, activated PI 3-kinase, and stimulated cellular proliferation, but did not enhance colony formation in soft agar (196). These studies also indicate that signal transduction pathways responsible for the mitogenic and transforming properties of the IGF-IR may be separable.

Figure 3 summarizes current information on the biological effects of different regions within the β-chain of the IGF-IR, as inferred by the mutational studies described above. Because, in contrast to studies on the insulin receptor, less extensive mutagenesis of the IGF-IR has been performed, further new insights into receptor function can be anticipated.

4. *Role of the insulin-like growth factor I receptor in signaling pathways activated by other growth factors, hormones, and oncogenes*

An intact IGF-IR appears to be required for at least some of the biological effects of both epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors (43, 52). When the human EGF receptor was overexpressed in IGF-IR-deficient mouse fibroblasts, EGF treatment did not stimulate DNA synthesis, cellular proliferation, or colony formation in soft agar above background levels (43). These defects were corrected by addition of the IGF-IR, and EGF became effective, even in the absence of IGF-I (43). Similarly, overexpression of the PDGF β-receptor in cells lacking the IGF-IR did not lead to ligand-activated proliferation or growth in soft agar (52). Again, the IGF-IR corrected both deficiencies (52). These experiments demonstrate that at least in fibroblasts, both the PDGF and EGF receptors depend on the IGF-IR for growth and transformation, and support the existence of interdependent or hierarchical signal transduction pathways mediated by these growth factors.

At least two dominant oncogenes also require an intact IGF-IR to transform fibroblasts. Large T antigen of simian virus 40 (SV40 T antigen) efficiently transforms IGFIR-expressing cells but cannot induce colony growth in soft agar or focus formation in its absence (186, 187, 211). An activated *ras* gene could stimulate proliferation, focus formation, and anchorage-independent growth when transfected into wild-type mouse fibroblasts, but had less effect on IGF-IR-deficient fibroblasts (186). For full transforming potential and for enhancement of cellular proliferation in serum-free medium, an intact IGF-IR is required (186, 187, 211), indicating that a functioning IGF-IR is needed for these oncogenes to be effective. The essential signaling pathways that the IGF-IR regulates for neoplastic transformation by these dominant oncogenes have not been eludicated.

The product of the *src* oncogene is a 60-kDa intracellular, membrane-associated tyrosine kinase that can stimulate cellular proliferation (155). Recent studies have shown that in fibroblasts transfected with a temperature-sensitive v-*src*, the IGF-IR is rapidly phosphorylated on tyrosine residues when the cells are incubated at the permissive temperature (155). *Src*-stimulated phosphorylation correlated with receptor activation, even in the ab-

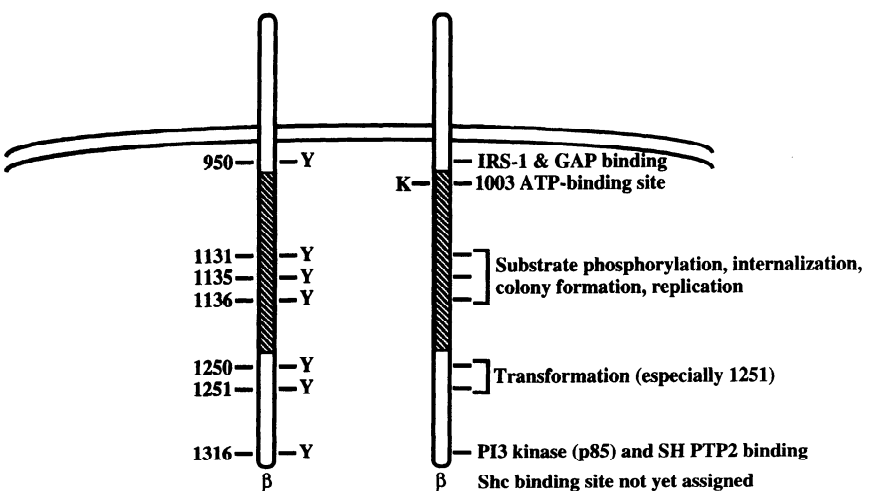

FIG. 3. Functional consequences of activation of IGF-I receptor β-subunit. Binding sites for intracellular signaling molecules are indicated. Assignment of different functions to specific phosphotyrosines is described in text. IRS-1, insulin receptor substrate 1; GAP, GTPase-activating protein; PI 3-kinase, phosphatidylinositol 3-kinase.

sence of IGF-I, and led to enhanced tyrosine phosphorylation of IRS-1 (155). These studies indicate that the IGF-IR may play a role in *src*-induced cellular transformation and suggest that one function of the protooncogene c-*src* is to modulate the activity of the IGF-IR. Further experiments will be required to test this hypothesis.

Growth factor-activated signaling pathways can be modified by signals from hormone receptors. Recent studies have demonstrated a role for receptors coupled to heterotrimeric GTP-binding proteins in modulating IGF-I action (55, 123, 164). The stimulation of MAP kinase phosphorylation and calcium entry into cells by IGF-I could be blocked by pertussis toxin (123, 198), implying a role for inhibitory G proteins (G_i) in regulating IGF-IR function. Overexpression in fibroblasts of a protein that binds $\beta\gamma$-G protein subunits also attenuated IGF-I-activated MAP kinase phosphorylation, but did not block induction by EGF (123), indicating that G_i proteins exert selective effects on tyrosine kinase receptors, and suggesting that $\beta\gamma$-subunits modulate stimulation of MAP kinases by IGF-I. The mechanisms through which G proteins modify IGF-IR function are unknown.

Thrombin activates a G protein-coupled receptor (55, 164). In vascular smooth muscle cells (VSMC), thrombin induces intracellular protein tyrosinc phosphorylation, activates c-*src* (164), and stimulates DNA synthesis and cellular proliferation (55). The IGF-IR β-subunit and IRS-1 are both rapidly phosphorylated on tyrosine residues in VSMC after thrombin treatment (164), and antisense oligonucleotides to the IGF-IR inhibited thrombin-induced DNA synthesis (55). Taken together, these observations suggest that the thrombin receptor may activate the IGF-IR through pathways involving c-*src* and G protein $\beta\gamma$-subunits. More direct evidence will be required to substantiate this hypothesis.

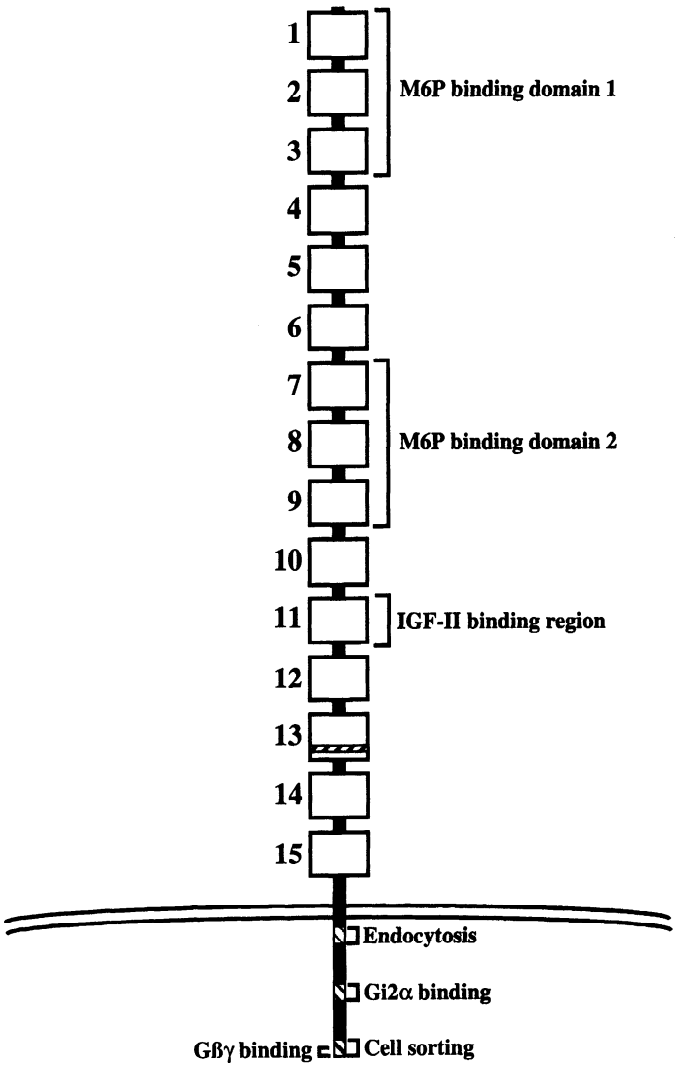

FIG. 4. IGF-II receptor. Fifteen repeating motifs comprising extracellular portion are depicted by boxes, and fibronection type II element in repeat 13 is identified by cross-hatching. Binding site for IGF-II and 2 regions that bind proteins containing mannose-6-phosphate (M6P) recognition motifs are indicated. Segments involved in receptor endocytosis and in sorting among different intracellular compartments are labeled, as are putative binding sites for subunits of heterotrimeric G proteins. See text for details.

B. Insulin-Like Growth Factor II Receptor

1. Structural considerations

The IGF-II receptor (IGF-IIR) is a single-chain membrane-spanning glycoprotein that also is known as the cation-independent mannose-6-phosphate receptor (106). The mature human receptor contains 2,451 amino acids that can be divided into three regions, a large 2,264-residue extracellular domain, a 23-amino acid transmembrane region, and a 164-residue carboxyl-terminal intracytoplasmic domain (138, 150) (Fig. 4). The extracellular part of the IGF-IIR binds ligands, and the intracytoplasmic region regulates movement among different cellular compartments. The human IGF-IIR gene has been mapped to chromosome 6q25-q27 (111) but has not been cloned, although the homologous 93-kb mouse gene on chromosome 17 is composed of 48 exons and codes for a predicted protein precursor of 2,482 amino acids (197).

The IGF-IIR is highly conserved among different species, with ~80% identity being found among bovine, rat, mouse, and human receptors (106). The homologous chicken protein is ~60% identical to the human IGF-IIR (232). The extracellular domain of each receptor is composed of 15 contiguous segments of 134–191 residues that share 16–38% identity (118, 124, 138, 197, 232). Each repeat contains eight cysteines in analogous positions and other conserved amino acids. Remarkably, all 118 cysteines in the extracytoplasmic region are conserved

among extracellular regions of human, mouse, and bovine receptors (197), and 120 of 122 cysteines found in the human receptor are present in the chicken protein (232). Sixteen potential sites of asparagine-linked glycosylation also are conserved among mammalian receptors (106). Repeating motif 13 additionally contains a 43-amino acid fibronectin type II repeat, originally identified in the collagen binding portion of fibronectin (118, 138, 150, 197, 203). Its function is unknown.

The entire extracellular region of the IGF-IIR has been found in serum and in cell-conditioned tissue culture medium (23, 101, 125), where it may play a role in the binding and transport of IGF-II (212). This soluble fragment arises by proteolysis (37), and the pathway regulating its appearance may represent a major mechanism of receptor removal from cells (37).

Each repeat of the human IGF-IIR shares 14–28% amino acid identity with the extracellular part of the cation-dependent mannose-6-phosphate receptor (CDMPR) (106). Both the CDMPR and IGF-IIR bind lysosomal enzymes and other proteins bearing a mannose-6-phosphate (M-6-P) recognition sequence (106), and both receptors transport lysosomal enzymes from their sites of synthesis to an endosomal/prelysosomal compartment (106). Only the IGF-IIR binds IGF-II, and this function appears to be limited to mammalian receptors (27, 36). Although both the IGF-IIR and CDMPR can be found in several subcellular compartments and bind similar proteins, only the IGF-IIR can bind M-6-P-containing ligands at the cell surface (106, 215). Thus the IGF-IIR is uniquely involved in the clearance of lysosomal enzymes from the extracellular environment.

2. *Insulin-like growth factor II receptor function*

As noted in section III*B1*, a major role for the IGF-IIR is to transport lysosomal enzymes from their sites of synthesis into an endosomal/prelysosomal compartment, and this function is shared with the CDMPR (106, 215). The importance of both receptors in the targeting of lysosomal enzymes has been illustrated in studies using mouse fibroblasts with genetic deficiencies in one or both proteins (122, 157). Cells lacking either receptor showed no upregulation of the remaining protein and had a ~50% decline in intracellular lysosomal enzyme levels, with an increase in their secretion (122, 157). The absence of both receptors caused massive secretion of newly synthesized hydrolases, a marked decrease in levels of intracellular lysosomal proteins, and impaired lysosomal function (122, 157). In fibroblasts missing a single receptor, the levels of secretion of individual lysosomal enzymes differed somewhat, depending on which receptor was absent, although neither receptor appeared to exert exclusive control over the transport of specific lysosomal proteins (122, 157). Taken together, these observations demonstrate that both M-6-P receptors are needed to ensure efficient targeting of lysosomal hydrolases.

The IGF-IIR is involved in regulating other M-6-P-containing proteins. The receptor plays a role in the uptake of thyroglobulin after its secretion by thyroid follicular cells and its subsequent degradation in lysosomes (87). The IGF-IIR also has been shown to bind the latent form of transforming growth factor-β1 (TGF-β1) through M-6-P residues located in its propeptide region (also known as the latency-associated peptide; reviewed in Ref. 71). It has been suggested that binding of the TGF-β1 precursor at the cell surface by the IGF-IIR is necessary for growth factor activation by cell-associated proteases, such as plasmin (71). The IGF-IIR additionally can bind proliferin, a putative murine growth factor (112), although the consequences of this interaction are unknown.

A third function for the IGF-IIR is the regulation of IGF-II action. Both genetic studies in mice and analyses in cultured cells have demonstrated a role for the IGF-IIR in growth factor clearance (102, 110, 217). In particular, mouse embryos engineered to lack receptors had elevated serum levels of IGF-II and showed accelerated somatic growth in the fetal period (110). Other experiments have presented the argument that the IGF-IIR can mediate signaling initiated by IGF-II binding (144, 148). These studies are discussed in section III*B2*.

3. *Structure-function relationships*

The extracellular part of the IGF-IIR encodes a single binding site for IGF-II and two sites for M-6-P-containing ligands (106). In contrast, the CDMPR has a single M-6-P binding site (106). The IGF-IIR binds IGF-II with high affinity (K_d 1–3 nM) but interacts minimally with IGF-I or insulin (106). Recent studies have localized the IGF-II binding site to repeat 11 of the rat, bovine, and human receptors (45, 77, 180) and have suggested that a 48-amino acid segment (residues 1508 to 1566 in the human receptor) comprises the minimal growth factor binding domain (180). This segment is poorly conserved in the chicken receptor (232), which does not bind IGF-II (27, 36). Binding sites for M-6-P-containing ligands have been mapped to repeating motifs 1–3 and 7–9 (220), and mutations in arginine residues 435 (domain 3) and 1334 (doman 9) of the bovine receptor were found to cause a dramatic decrease in binding (44). Figure 4 summarizes these data.

The intracytoplasmic part of the IGF-IIR encodes segments responsible for sorting among different subcellular compartments (94, 119), for endocytosis (119), and potentially for coupling to inhibitory GTP-binding proteins (specifically $G_{i\text{-}2}$) (144, 148) (Fig. 4). In addition, the intracellular part of the receptor contains residues phosphorylated by several protein kinases (134, 175), but the role of phos-

phorylation in IGF-IIR function is unknown. The signal for efficient sorting of newly synthesized lysosomal enzymes has been mapped to the carboxyl-terminal of the receptor, with the minimal signal comprising the last four amino acids (94), while the region required for internalization from the plasma membrane has been localized to a conserved hexamer within the juxtamembrane domain: Tyr Lys Tyr Ser Lys Val (27, 91). Mutant receptors with tyrosine to alanine substitutions at this site are not internalized and accumulate at the cell surface (27).

Ten to twenty percent of IGF-II receptors are located at the plasma membrane and are in equilibrium with intracellular receptors. Cycling of receptors between different compartments is rapid and constitutive (147, 215), but partitioning can be regulated, since treatment of rat adipose cells with insulin (6, 121), or human skin fibroblasts with insulin, IGF-I, IGF-II, EGF, or M-6-P (24) was found to cause a rise in the fraction of surface receptors without changing their total number. The increase in membrane-associated IGF-II receptors stimulated by M-6-P could be blocked by pretreatment of cells with pertussis toxin or cholera toxin, implying regulation by both stimulatory and inhibitory GTP-binding proteins (24), although these toxins could not inhibit growth factor-mediated receptor redistribution (24). The mechanisms by which these agents influence receptor trafficking are unknown.

While a number of studies have presented evidence implicating the IGF-IIR in the removal of IGF-II from the extracellular environment (106, 110, 217), a direct role for the receptor in mediating IGF-II action remains controversial (89, 105, 144, 148). As noted above, genetic studies have not supported a signaling function for the IGF-IIR (110, 217), and cell biological studies also generally have been negative. An IGF-IIR antibody that inhibited growth factor binding did not block IGF-II-stimulated amino acid and glucose uptake in L6 myoblasts, and IGF-II analogues with reduced affinity for the IGF-IIR but normal affinity for the IGF-IR have been shown to be active in cultured cells (68, 102, 177). These latter results suggest that the IGF-IR mediates the biological effects of IGF-II. On the other hand, it has been demonstrated that IGF-II treatment stimulates glycogen sythesis in liver cells, an effect that could be mimicked by antibodies to the IGF-IIR (84). Other studies also have supported the hypothesis that IGF-II can act through the IGF-IIR. For several years, Nishimoto and colleagues (104) have used a biochemical approach in investigating the potential role of the IGF-IIR in IGF-II signaling. In initial studies, they found that IGF-II could induce calcium influx and promote DNA synthesis in EGF-treated and PDGF-primed mouse fibroblasts (104). These effects could be reproduced by an anti-IGF-IIR antibody and could be blocked by pretreatment of cells with pertussis toxin, implying that an inhibitory GTP-binding protein was functionally coupled to the IGF-IIR following stimulation by IGF-II (144, 148). Subsequent studies, using purified components reconstituted in phospholipid vesicles, suggested the involvement of $G_{i\text{-}2}$ proteins in IGF-IIR function (144), and a 14-amino acid segment of the intracytoplasmic region of the receptor was later shown to interact with purified $G\alpha_{i\text{-}2}$ (148, 149). A more recent report has suggested that the COOH-terminal tail of the IGF-IIR may bind $\beta\gamma$-subunits (89).

In marked contrast to these data are recent results from Korner et al. (105), who transfected wild-type and mutated receptors into IGF-IIR-deficient fibroblasts. Neither full-length receptors nor receptors lacking the 14-amino acid putative G protein coupling region mediated IGF-II-stimulated activation of $G_{i\text{-}2}$ proteins in transfected cells or in reconstituted vesicles (105). While these opposite results may reflect differences in experimental design, a role for the IGF-IIR in growth factor signaling remains unproven.

C. Insulin-Like Growth Factor Binding Proteins

1. *Structural considerations*

The six IGFBPs characterized to date comprise a structurally related family of secreted proteins that bind both IGFs with high affinity (9, 19, 95). The IGFBPs vary in length from 216 to 289 amino acids and are composed of shared cysteine-rich amino- and carboxyl-terminal domains (9). In contrast, the central portion of each IGFBP is unique (9).

The IGFBP genes have a simple and conserved structure, and each gene is transcribed and processed into one or at most a few mRNAs (9). The genes encoding IGFBP-1, -2, -4, -5, and -6 contain four exons, and the IGFBP-3 gene has five exons (4, 9, 108). Exon-intron junctions interrupt coding regions at similar locations in all six genes (108). Two pairs of IGFBP genes are clustered in a tail-to-tail configuration in the human and mouse genomes (4, 66, 107). The IGFBP-1 and IGFBP-3 genes are located 20 kb apart on human chromosome 7p14-p12 (66), and IGFBP-2 and IGFBP-5 are found on 2q31-q34 (4). In the mouse, this latter pair is separated by 5 kb (107). In contrast, the human IGFBP-4 gene is located on chromosome 17q12-q21, and IGFBP-6 is on chromosome 12 (95, 204).

2. *Insulin-like growth factor binding proteins have multiple functions*

The IGFBPs are modulators of IGF action (Tables 2 and 3). The IGFs present in the blood and other biological fluids are bound to IGFBPs, and this interaction maintains reservoirs of these growth factors in the circulation and elsewhere (17, 95). In conjunction with another protein, termed the acid labile subunit (ALS), IGFBP-3 is primarily

TABLE 2. *Actions of IGF binding proteins*

To transport IGFs from the circulation to peripheral tissues
To maintain a reservoir of IGFs in the circulation
To potentiate or inhibit IGF action
To mediate IGF-independent biological effects

responsible for maintaining IGF levels in the blood (17, 114). Other IGFBPs found in the bloodstream, including IGFBP-1, IGFBP-2, and IGFBP-4, can cross endothelial barriers and thus may transport IGFs from the circulation to peripheral tissues (9, 17, 95). Several IGFBPs are found in the extracellular environments of many tissues and may regulate IGF accessibility to receptors and/or provide a local storage depot. These local functions of IGFBPs may be modulated by interactions with the extracellular matrix and with the cell surface. In addition, local levels of several IGFBPs are modified through specific proteolysis. Thus the dynamic interactions between IGFs and their binding proteins regulate IGF availability and, ultimately, IGF action.

The roles of individual IGFBPs in growth and development have not been examined in detail, although a wealth of studies exist in tissue culture systems, as summarized recently (17, 95). Transgenic mice overexpressing IGFBP-1 showed mild growth retardation and modest hyperglycermia (163); mice lacking IGFBP-2 by targeted gene disruption are reported to be normal (223); and overexpression of IGFBP-3 caused selective increases in heart, liver, and spleen weights (141). Manipulations of other IGFBP genes in animals have not been described. Because it is likely that several of the proteins share identical functions, deficiency of multiple IGFBPs may be required to reveal developmentally significant abnormalities.

IV. PHYSIOLOGICAL ACTIONS OF INSULIN-LIKE GROWTH FACTORS I AND II

A. Roles in Somatic Growth

1. Postnatal growth

The central role of IGF-I in regulating postnatal growth and in mediating the growth-promoting effects of GH has been confirmed in multiple ways (reviewed in Ref. 95). Pituitary-deficient rats did not gain weight or grow in length unless administered GH or IGF-I (82, 181, 189), and GH treatment stimulated IGF-I expression (40, 95, 178). In addition, mice with genetic IGF-I deficiency showed impaired growth (117, 158).

The effects of GH or IGF-I on rates of growth of individual organs and in the whole animal are not identical. Although systemic administration of each protein to pituitary-ablated male rats led to identically enhanced longitudinal bone growth in one study (82), IGF-I stimulated a greater increase in kidney, spleen, and thymus weight than GH (82, 189). These qualitatively different responses to GH and to IGF-I can be explained by the fact that GH induces synthesis of IGF-I in multiple tissues (95, 176), and also enhances expression of the major serum carrier protein IGFBP-3 and its cofactor, ALS, in the liver (46), thus leading to restoration of the stable circulating ternary complex containing these two proteins and IGF-I (9, 46). The net consequence of these effects may be more sustained exposure of all tissues to IGF-I, leading to a more prolonged growth response. In contrast, although IGF-I can stimulate expression of IGFBP-3 (9, 26), it has no effect on ALS production and may even reduce the contribution of GH to ALS synthesis and secretion by feedback inhibition of GH production in the pituitary (26).

As expected, overexpression of bovine, murine, or rat GH caused increased growth in transgenic mice (129, 152). Mature animals were 1.6–1.9 times larger than control littermates and had two- to threefold elevations in serum IGF-I concentrations (129, 152). Despite a >10-fold rise in circulating levels of GH beginning at birth, accelerated growth did not commence until 2 wk of age, when serum IGF-I levels first became elevated compared with control mice (129). This delay in the response to GH probably reflects the ontogeny of the GH receptor and its associated signal transduction pathways, which do not appear until this time (127, 202).

Transgenic mice expressing human IGF-I in the liver and other tissues also showed enhanced growth (20, 51, 128), while mice with IGF-II transgenes did not (174, 213, 218, 222). A 1.3-fold increase in weight occurred in one transgenic line in which serum IGF-I levels were 1.5 times above controls, although this was manifested as selective organomegaly rather than as an increase in skeletal size (128), a result consistent with the mechanism of differential growth responses outlined above. In contrast, overexpression of human IGF-II led to little change in weight or

TABLE 3. *Actions of individual IGF binding proteins*

IGFBP	Potential Functions	Cell Surface/ Matrix Binding	Modification by Proteolysis
1	Minor serum carrier; potentiates/ inhibits IGF action	Yes	No
2	Minor serum carrier; potentiates/ inhibits IGF action	No	Yes
3	Major serum carrier; potentiates/ inhibits IGF action; IGF-independent modulator of cell proliferation	Yes	Yes
4	Inhibits IGF action	No	Yes
5	Potentiates/inhibits IGF action	Yes	Yes
6	Potentiates/inhibits IGF action?	No	Possibly

length compared with nontransgenic controls, despite up to an eightfold rise in circulating growth factor levels (213, 218, 222). Adult transgenic mice with greater elevations in serum IGF-II concentrations (20- to 30-fold) were even smaller than controls, primarily because of major reductions in fat mass secondary to the metabolic effects of massive growth factor overexpression (174). Lack of a positive effect on growth also was seen in pituitary-ablated rats administered IGF-II (80). These results, together with observations showing that genetic IGF-II deficiency in mice did not alter growth rates after birth (53), confirm the minimal role that IGF-II plays in somatic growth during the postnatal period in rodents. Although similar experiments have not been performed in larger mammals, it is likely that IGF-II also has a minor effect on postnatal growth in these species.

Despite the lack of efficacy of IGF-II in enhancing somatic growth during the postnatal period in mice and rats, specific tissues were stimulated when selective growth factor overexpression was achieved (174, 213, 218, 222). Transgenic mice in which IGF-II had been targeted to the skin and uterus showed marked tissue hyperplasia, but little change in whole body growth rates, despite up to a threefold increase in circulating IGF-II levels (218). Similar local growth-promoting effects were found when IGF-II was expressed in the thymus (213). Taken together with studies noted above, these results demonstrate that IGF-I and IGF-II can individually contribute to tissue overgrowth, yet do not explain why IGF-II cannot substitute for IGF-I in maintaining normal somatic growth in rodents during the postnatal period.

2. *Embryonic and fetal growth*

A) ROLE OF IGF-II AND THE IGF-IIR. The postulated function of IGF-II as a key fetal growth factor (49, 139) was proven when a growth-deficiency phenotype was created in mice by targeted disruption of the IGF-II gene (53, 54). Mice lacking IGF-II were ~60% the size of wild-type littermates at birth (53, 117), and diminished growth was apparent as early as embryonic *day 11* (10). As noted above, a normal growth rate was observed after birth, and the animals were otherwise viable and fertile (53, 54). Equivalently impaired fetal growth was found in homozygous mutant mice and in heterozygotes inheriting a paternally derived mutant allele (53, 54), and additional studies demonstrated that in most tissues IGF-II mRNA was produced only from the paternal chromosome (54). Thus IGF-II gene expression is regulated by parental imprinting, an epigenetic modification that by unknown mechanisms controls gene expression (11, 65). To date, only a small number of genes have been shown to be imprinted, and many of these are involved in growth and development (reviewed in Refs. 11, 65). More recent studies have established that the human IGF-II gene also is imprinted (67, 78, 216) and have shown that an adjacent imprinted locus, H19, which is expressed from the maternally derived chromosome in mice and in humans (11, 65), may modulate the expression of the IGF-II gene in mice (113).

Despite its unproven status as a signaling receptor, the IGF-IIR also is critical for normal embryonic and fetal growth in rodents (110, 217). In mice, the IGF-IIR gene has been mapped to an imprinted locus on chromosome 17, termed T-associated maternal effect (Tme) (11, 12). An active, maternally derived Tme chromosome was shown to be essential for viability (12, 70). Targeted deletions of the mouse IGF-IIR gene confirmed that receptor deficiency contributed to the Tme phenotype and proved that the gene was expressed principally from the maternal chromosome (110, 217). Mice lacking IGF-IIR expression died in the perinatal period with major cardiac anomalies (110, 217) and manifested a growth advantage (125–130% of the weight of wild-type littermates; Refs. 110, 217) that was accompanied by up to a 2.6-fold rise in circulating levels of IGF-II (110). Because mice with Tme or IGF-IIR deficiency were variably rescued in genetic crosses with animals lacking IGF-II (70, 217), it seems likely that overaccumulation of IGF-II is a manifestation of the Tme phenotype and that one major role of the IGF-IIR in the embryo and fetus is to limit the biological effects of IGF-II. To date, no equivalent data exist in other mammalian species, and the IGF-IIR may not be imprinted in humans (97).

B) ROLE OF IGF-I AND THE IGF-IR. As expected, mice lacking IGF-I showed markedly diminished postnatal growth (10, 158), but also had impaired fetal growth and development (117, 158). Depending on the study, mice heterozygous for a disrupted IGF-I gene were 80–100% the size of wild-type littermates at birth (10, 158), while homozygous mutant animals were ~60% of normal weight and length (117, 158). A decline in growth rate beginning at embryonic *day 13.5* was noted in homozygotes (10). The IGF-I-deficient mice additionally showed diminished viability, with perinatal death being attributed to muscle hypoplasia and decreased maturation of the lungs (117, 158). There is no evidence that the IGF-I gene is imprinted.

As would be anticipated from its central role in IGF signaling, the IGF-IR is necessary for normal embryonic and fetal growth (10, 117). Although heterozygous animals were normal, mice lacking the IGF-IR were small at term, ~45% of wild-type size, and were inviable, dying of respiratory failure (117). The pups additionally had marked muscle hypoplasia, with diminished numbers of myocytes in all skeletal muscle groups, severe skin hypoplasia, delayed bone ossification, and decreased numbers of oligodendocyte precursors in the central nervous system (117). The IGF-IR gene is not imprinted.

Mice lacking both IGF-I and IGF-II, or animals defi-

cient in both IGF-II and the IGF-IR, had an exacerbated dwarf phenotype and were ~30% of wild-type size at term (117). These mice died at birth of lung failure and had many of the same anatomic defects as were found in IGF-IR mutants (117). Taken together, these results demonstrate that expression of each IGF and each receptor is required for normal embryonic and fetal growth and indicate that there is little compensatory upregulation of one component when another is absent. Table 4 summarizes the current view of the genetics of the IGF system in mouse development.

B. Role in Cellular Proliferation

The Gap1 (G_1) phase of the cell cycle is the interval when cells prepare for DNA synthesis (S phase), which is marked by replication of the chromosomes. Studies in mouse fibroblasts have demonstrated roles for several growth factors in progression of the cell cycle from G_1 to S (153). A pulse of PDGF is required for fibroblasts to become competent to move into G_1, and either EGF or IGF-I was found to be necessary for subsequent progression into S phase (16, 153). Pardee (153) also identified a point in G_1 termed V, which was operationally defined as the halting stage observed in the absence of essential amino acids. In addition to amino acids, which are necessary for protein synthesis, only IGF-I was shown to be required for cells to move past V into S phase (16, 153).

A wide variety of cell types demonstrate a mitogenic response to IGF-I. As summarized in a recent review (95), cells as diverse as keratinocytes, osteoblasts, smooth and skeletal muscle cells, renal mesangial cells, thyroid follicular cells, chondrocytes, mammary epithelial cells, erythroid precursors, and many others all showed enhanced proliferation after IGF-I treatment. More critically, recent observations have demonstrated that a functioning IGF-IR is needed for many aspects of normal cell cycle progression (186). In fibroblasts derived from mouse embryos with a null mutation in the IGF-IR, all phases of the cell cycle were slowed, and doubling time after incubation in medium with 10% serum was prolonged from 44 h in wild-type fibroblasts to 109 h in cells from receptor-deficient mice (186). In addition, cell cycle progression could not be stimulated to a normal rate in these fibroblasts by expression of dominant oncogenes (186, 187). These in vitro results potentially provide an explanation for the marked growth retardation seen in mouse embryos lacking the IGF-IR (117), since delayed cell replication and consequently diminished cell number could be responsible for the dwarfism that accompanies this mutation. Because mouse embryos lacking IGF-IIR expression are larger than wild-type littermates (110, 217), it will be of interest to determine if the cell cycle is commensurately shortened in fibroblasts from these pups.

TABLE 4. *Genetics of the IGF system in mouse development*

Locus	Function	Deficiency	Imprinting
igf-1	Somatic growth	Dwarfism	No
igf-2	Somatic growth	Dwarfism	Paternal expression
igf-1R	Somatic growth, muscle, bone, nerve, and skin development	Dwarfism, neonatal death	No
igf-2R	Lysosomal enzyme sorting, IGF-II clearance	Fetal overgrowth, late fetal/ neonatal death	Maternal expression
igfbps1-6	Serum transport, modulation of IGF actions	?	No?

C. Roles in Development, Differentiation, and Cell Death

1. *Insulin-like growth factor action during development in vivo*

Similar anatomic defects were seen in mice lacking the IGF-IR, the two IGFs, or both IGF-II and the IGF-IR (117). These pups all had a generalized growth impairment accompanied by severe skeletal muscle hypoplasia, marked skin hypoplasia, delayed bone ossification, and changes in the cellular architecture of the spinal cord and brain, potentially secondary to diminished numbers of nonneuronal cells (117). As described below, IGFs exert effects on each of these tissues.

2. *Insulin-like growth factors and skeletal muscle*

A) IGF ACTION IN VIVO. In direct contrast to the diminished muscle mass seen in mice lacking IGF-I or the IGF-IR (117, 158), overexpression of IGF-I in skeletal muscle of otherwise normal transgenic mice was found to stimulate myofiber hypertrophy (41). Similar results were observed with isolated chicken muscle cells placed in a three-dimensional collagen gel matrix and incubated with IGF-I (214). The IGF-I mRNA and protein have been detected in newly replicating rat skeletal myoblasts after ischemic or toxic injury (29, 64, 93), and IGF-I and IGF-II gene expression were both induced as early events during work-induced muscle hypertrophy in pituitary-intact and hypophysectomized rats (60). These latter experiments implicate the two IGFs in muscle growth and regeneration in response to locally produced stimuli.

B) IGF ACTION IN CULTURED MYOBLASTS. The muscle hypoplasia seen in IGF-IR- and IGF-I-deficient mice (117, 158) could be secondary to diminished myoblast replica-

tion, to decreased differentiation, or to increased muscle cell death. In cultured myoblasts, IGF-I and IGF-II have been shown to stimulate a variety of anabolic effects that generally culminate in enhanced differentiation (reviewed in Ref. 72). Like other growth factors, the IGFs can stimulate myoblast replication, but also paradoxically and uniquely can induce terminal differentiation (reviewed in Ref. 72). The mechanism of this latter action is not completely known but may be linked to the ability of IGFs to induce expression of the myogenic regulatory factor myogenin (73). In this regard, overexpression of IGF-I, IGF-II, or the IGF-IR in cultured myoblasts all have been shown to lead to accelerated differentiation (41, 160, 193), although in other experiments, IGF-II overexpression potentiated anchorage-independent muscle cell growth and impaired differentiation (135). These different effects of enhanced IGF-II production on myoblasts will require further analysis.

Because IGF-II is produced by most myoblast lines as they differentiate (reviewed in Ref. 72), it seems reasonable to postulate that the IGF system participates in a "feed-forward" loop that may modulate the rate and extent of terminal differentiation. This loop in turn could be modified by IGFBPs, which appear to be able to blunt IGF action in muscle cells (8, 30, 92, 188). Another role for endogenously secreted IGF-II may be to minimize cell death during the transition from proliferating to differentiating myoblasts. In recent studies, our laboratory has generated myoblast cell lines in which IGF-II production was inhibited (194). These cells proliferated normally in rich medium, but underwent rapid apoptotic death during incubation in differentiation medium (194). Muscle cell death could be prevented by addition of IGF-I or IGF-II to differentiation medium, and also could be blocked by the protein synthesis inhibitor cycloheximide (194). Similar observations regarding the pivotal role of IGF-II in preventing apoptotic cell death have been made in myoblast cell lines derived from mice with muscular dystrophy and also have been seen directly in dystrophic muscle (190). Given these results, it is thus possible that IGF action is essential for muscle cell survival and that the muscle hypoplasia seen in the absence of normal growth factor signaling (117, 158) results from enhanced cell death.

3. *Insulin-like growth factors and skin*

As described in section IV*A2*B, IGF-IR or IGF deficiency caused marked skin hypoplasia in mice, with deficits in the stratum spinosum layer of the epidermis and in the number of hair follicles (117), while transgenic mice with IGF-II overexpression in the epidermis showed overgrowth of both skin and hair (218). Because proliferation of primary human keratinocytes has been demonstrated to be induced by IGF-I (13), and because IGF-I is produced by dermal fibroblasts (13), IGF action in the skin during normal development may be by a paracrine mechanism.

4. *Insulin-like growth factors and bone*

A characteristic feature of GH-deficient animals is impaired long bone growth, which can be restored to normal by treatment with GH or IGF-I (82, 181). In addition, mouse embryos lacking IGF-IR expression showed up to a 2-day delay in ossification within the skeleton (117). Thus IGF action is essential for normal bone growth and maturation in vivo.

Insulin-like growth factor I produces a variety of effects on bone cells in culture. Growth factor treatment enhances proliferation of preosteoblastic cells and stimulates synthesis of one of the major components of bone matrix, type I collagen (56). These effects on bone may be mediated through autocrine, paracrine, and endocrine mechanisms (56). Skeletal tissue, including matrix, is a rich source of growth factors, including IGFs and IGFBPs (56). In addition, osteoblastic cells synthesize IGF-I and several IGFBPs (130), and IGF-I expression has been shown to be modulated by a number of hormones, cytokines, and growth factors that affect bone growth (56, 130), implying a complex relationship between regulation of IGF-I secretion and action within the skeleton.

5. *Insulin-like growth factors and the nervous system*

The major morphological alteration found within the central nervous system of IGF-IR-deficient mice was a marked diminution in nonneuronal cells, and primary cultures from embryonic forebrains revealed a decline in the number of recognizable oligodendrocyte precursors (117). This is not surprising, since IGF-I has been identified as a potent inducer of rat cerebral oligodendrocytes in culture (131) and has been shown to act as a survival factor for glial progenitor cells and for oligodendrocytes isolated from the developing rat optic nerve (14, 161). Because oligodendrocytes are most vulnerable during the first 3 days after their production from precursor cells (161), the absence of IGF action could be predicted to result in a loss of these cells within the developing brain and spinal cord (117).

Insulin-like growth factor I additionally influences the viability of different types of neurons and related cells (61, 61a, 62) and enhances neuronal differentiation (151). Treatment with IGF-I stimulated mitogenesis of sympathetic neuroblasts in primary culture (61) and acted as a survival factor for adrenal chromaffin cells, a neural crest derivative ontogenetically related to sympathetic neurons (75). Insulin-like growth factor I also has been found to maintain murine embryonic neuroepithelial cells in primary culture (62) and to inhibit apoptotic cell death in-

duced when cultured cerebellar granular neurons are incubated with low levels of potassium (61a).

Insulin-like growth factors I and II additionally may act as muscle-derived neurotrophic factors (28–30). The IGF-II mRNA levels in muscle are reciprocally related to innervation (90). The IGF-I gene expression also is induced in denervated muscle, and locally produced IGF-I can stimulate intramuscular neurite outgrowth and intramuscular interstitial cell (fibroblast) proliferation (29, 30). Insulin-like growth factors thus may function to promote local restorative effects in denervated muscle.

6. *Insulin-like growth factor action and the prevention of cell death*

Insulin-like growth factors I and II have been shown to function as survival factors for other cell types in addition to myoblasts, neurons, and oligodendrocytes. Insulin-like growth factor II has been identified as the growth factor required for full tumorigenesis in transgenic mice expressing SV40 T antigen in the islets of Langerhans (33, 34). In the absence of IGF-II action, these cells showed both an enhanced rate of death and reduced tumor formation (33, 34). Both IGF-I and PDGF have been found to blunt apoptosis induced by c-Myc in serum-deprived fibroblasts, and this effect was independent of cell cycle progression or ongoing protein synthesis (85). In other studies, IGF-I and the IGF-IR were shown to be required for survival of cultured hematopoietic cells after trophic factor withdrawal (173), to prevent apoptosis in fibroblasts exposed to the topoisomerase inhibitor etoposide (85, 185), and to block the death of a variety of tumor cell lines cultured for short term in vivo (167, 168). From a more physiological perspective, IGFs also may be responsible for the prevention of apoptosis by gonadotropins during ovarian follicular development (35), an effect consistent with other roles for IGF-I and IGF-II as mediators of gonadotropin action (2, 79). One general conclusion that emerges from these various observations is that IGF action can prevent the premature death of many cell types, a conclusion consistent with the marked cellular hypoplasia in tissues of mice lacking a functioning IGF-IR (117).

D. Roles in Cancer

1. *Insulin-like growth factor I receptor in tumorigenesis*

As noted in section IIIA3, massive overexpression of the IGF-IR in fibroblasts leads to tumor formation when transfected cells are injected into immunodeficient nude mice (96). This transforming effect can be blocked by expression of truncated receptors lacking a tyrosine kinase domain (86, 116, 159), possibly through the formation of heteromeric receptors containing both wild-type and mutant $\alpha\beta$-heterodimers. Fibroblasts lacking the IGF-IR also do not form tumors in nude mice (120, 186), nor do cells expressing receptors with mutations in the tyrosine cluster at amino acids 1131, 1135, and 1136 (81, 86, 100), or the carboxyl-terminal tyrosine-1251 (21, 137), thus indicating that intact receptors are required to mediate tumorigenesis (Fig. 3).

C6 rat glioblastoma cells form rapidly progressing malignant neoplasms when injected into immunodeficient mice or syngeneic rats (168, 169, 206). Cells pretreated with antisense oligonucleotides to the IGF-IR formed smaller tumors than controls (169). A decline in tumor cell survival in vivo also was observed in C6 glioblastomas stably expressing an IGF-IR antisense cDNA (167). In both experimental systems there was a direct correlation between diminished tumorigenesis and a decline in IGF-IR expression. In addition, cells lacking the IGF-IR appeared to die by apoptosis (167, 168). These results, in conjunction with observations described earlier, point to several functions for the IGF-IR in experimental cancers, including stimulation of cellular proliferation, prevention of programmed cell death, and collaboration with other growth factor signaling pathways and oncogenes in mediating transformation.

Depsite the impressive evidence supporting a role for the IGF-IR in experimental carcinogenesis, there are only correlative data demonstrating the importance of the receptor in human cancers. Expression of IGF-IR appears to be enhanced in Wilms tumors and breast cancers compared with adjacent normal tissue, and both squamous and small cell lung cancers have high levels of receptors, as do many other neoplasms (reviewed in Ref. 115). However, no studies have been performed yet that directly implicate the IGF-IR in the initiation or propagation of human cancers, nor have any therapies been initiated to test the hypothesis that IGF-IR function is critical for tumorigenesis in humans.

2. *Insulin-like growth factors I and II in cancer*

C6 glioblastoma cells produce IGF-I (206), and as noted above, are tumorigenic when injected into syngeneic rats (169, 206). Cells stably expressing an IGF-I antisense cDNA did not form tumors in rats, and surprisingly, caused regression of preexisting neoplasms (206, 208). Although the mechanism of tumor regression is not known, it may be related to alterations in immunogenicity triggered by the antisense cells (207).

Overexpression of IGF-II has been found in precancerous liver nodules in viral-induced experimental hepatic cancers (179, 226) and during late-progression stages of hepatocarcinogenesis in IGF-II transgenic mice (174, 179).

Similarly, IGF-II is required for tumorigenesis of pancreatic β-cells in transgenic mice expressing SV40 T antigen under control of an insulin gene promoter (33, 34), as noted in section IV*C6*. In this model, IGF-II is needed both for survival and for enhanced proliferation of preneoplastic cells (34), and activation of maternal and paternal copies of the IGF-II gene accompanies tumor formation (33), indicating that loss of imprinting may be required for tumorigenesis. A similar phenomenon has been observed in human Wilms tumors, where IGF-II overexpression has been found (166, 183), and both IGF-II alleles become activated (145, 162). Loss of imprinting of the IGF-II gene also has been described in human rhabdomyosarcomas and lung cancers (231). Because in several of these human cancers, IGF-IR levels are also elevated, as described above, it is possible that an IGF autocrine or paracrine loop becomes selectively activated during tumorigenesis.

3. *Does the insulin-like growth factor II receptor contribute to neoplastic transformation?*

Recent reports have indicated that IGF-IIR expression is reduced in hepatocellular carcinomas (57), and loss of heterozygosity at the human IGF-IIR locus has been found in DNA samples from ~70% of liver cancers (57). In ~25% of these tumor DNAs, inactivating mutations have been identified in the remaining IGF-IIR allele (58). These results potentially implicate the IGF-IIR as a tumor suppressor for liver cancer, possibly through its effects on the clearance and degradation of IGF-II and/or on the activation of TGF-β1, a known growth inhibitor (71). These intriguing initial observations will require substantiation and the development of experimental models to test this hypothesis directly.

4. *Is insulin-like growth factor binding protein 3 an antineoplastic agent?*

In addition to its identification as a serum carrier protein for IGFs (9), IGFBP-3 was independently purified and characterized from medium conditioned by NIH-3T3 cells, based on its growth inhibitory properties (22). The inhibitory effects of IGFBP-3 on cellular proliferation are manifested in fibroblasts that either express or lack the IGF-IR (39, 210), thus indicating that growth suppression can occur by mechanisms independent of IGF-IR action. In recent studies, the effects of TGF-β2 on inhibiting growth of a cultured human breast cancer cell line have been shown to be mediated by IGFBP-3 (146), and IGFBP-3 has been identified as a target gene regulated by the growth suppressor p53 (25). Taken together, these results suggest a role for IGFBP-3 as an anticancer factor and point toward the possibility that the IGFBP-3 gene on chromosome 7p14-p12 may be a tumor suppressor locus.

5. *Insulin-like growth factor II and tumor hypoglycemia*

The secretion of IGF-II by certain large tumors, particularly fibrosarcomas, rhabdosarcomas, and leiomyosarcomas, has been shown to cause tumor-associated hypoglycemia that can be severe in its clinical manifestations (reviewed in Ref. 47). One puzzling aspect of the pathophysiology of this condition is that total serum levels of IGF-II in these patients often are not elevated, and may even be reduced (47, 228). This may be explained by marked abnormalities in IGFBPs that accompany this condition (18, 228). The ternary complex, consisting of IGFBP-3, ALS, and IGF-I or IGF-II, is virtually absent from the circulation of these individuals, although IGFBP-3 is present (18). Normally, this complex prevents passage of its bound IGFs through the capillary membrane, while other IGFBPs are more permeable (9, 17, 95). Thus, in the absence of normal serum carrier proteins, IGFs have greater access to peripheral tissues. The etiology of the decline in abundance of ALS also may be secondary to enhanced IGF-II action, since through feedback inhibition of GH secretion by the pituitary, ALS production is suppressed (47).

V. SUMMARY AND PERSPECTIVES ON FUTURE RESEARCH

During the last five years, an explosion of new information has confirmed and extended our understanding of the pleiotropic effects of the IGF system on growth, development, and intermediary metabolism. Through in vivo gene manipulations in mice, it has been established that IGF-I and IGF-II and their two receptors are essential for normal embryonic and fetal growth. These and other studies also revealed that the genes encoding IGF-II and the IGF-IIR are regulated by parental imprinting, an epigenetic modification that by unknown mechanisms controls gene expression. Insulin-like growth factor II is expressed primarily from the paternally derived chromosome, and the IGF-IIR from the maternally derived allele. Loss of imprinting and biallelic expression of IGF-II additionally has been demonstrated to accompany certain cancers. Thus understanding the molecular basis of imprinting of these genes will have far-reaching consequences for both biology and medicine.

In mice, IGF-IR deficiency caused marked growth impairment and contributed to the hypoplasia of several tissues, while in fibroblasts lacking the receptor, all phases of the cell cycle were slowed. These results illustrate the importance of intact receptor-mediated signaling pathways for normal cell proliferation. Additional structure-function studies have established that tyrosine kinase activity is essential for all biological effects regulated by the

IGF-IR and have revealed distinct roles for subsets of autophosphorylated tyrosines in receptor function. These results provide an impetus to elucidate the specific biochemical pathways through which the IGF-IR controls both mitogenesis and cellular transformation.

Recent studies have suggested that the IGF-IIR and IGFBP-3 both may function as tumor suppressors. Inactivating mutations of the IGF-IIR were found in hepatocellular carcinomas, and IGFBP-3 was identified as an IGF-independent growth inhibitor and as a target for the growth suppressor p53. It will be of great interest to establish the roles of these two proteins in preventing tumorigenesis and to determine the mechanisms of inactivation of the IGF-IIR in cancer.

In summary, the many recent advances in the basic biology of the IGF system described in this review provide renewed opportunities to understand how these growth factors, their receptors, and binding proteins regulate cell and tissue survival, growth, and differentiation. The next decade should be as exciting for IGF research as has been the last.

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