Endocytosis of Insulin-like Growth Factor II by a Mini-receptor Based on Repeat 11 of the Mannose 6-Phosphate/Insulin-like Growth Factor II Receptor*

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The mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF-II receptor) plays an important role in controlling the extracellular level of the insulin-like growth factor II (IGF-II) by mediating its binding at the cell surface and delivery to lysosomes. Loss of the receptor is associated with an accumulation of IGF-II, which can cause perinatal lethality if it is systemic, or local proliferation and tumorgenesis if it is spatially restricted. The extracytoplasmic domain of the receptor consists of 15 homologous repeats, of which repeat 11 carries the IGF-II-binding site of the multifunctional receptor. To investigate whether repeat 11 is sufficient to mediate binding and internalization of IGF-II, a construct consisting of repeat 11 fused to the transmembrane and cytoplasmic domain of the M6P/IGF-II receptor was transfected into mouse embryonic fibroblasts. The construct was expressed as a stable membrane protein which binds IGF-II with a 10-fold lower affinity as observed for the M6P/IGF-II receptor and is found at the cell surface and in endosomes. It mediates the internalization of IGF-II and its delivery to lysosomes, suggesting that it can function as a IGF-II mini-receptor controlling the extracellular IGF-II level.

The mannose 6-phosphate/insulin-like growth factor II (M6P/IGF-II) receptor is a type I transmembrane protein with an apparent molecular mass of 300 kDa which cycles between endosomes and the TGN as well as between endosomes and the cell surface (for reviews, see Refs. 1–3). The extracytoplasmic domain is composed of 15 repeating units with an average length of 147 amino acids and an overall identity of 26 (4). The extracytoplasmic domain contains two binding sites for mannose 6-phosphate located in repeats 3 and 9. In both domains, a conserved arginine residue is critical for binding of mannose 6-phosphate (5). Through a separate binding site located in repeat 11 the M6P/IGF-II receptor binds IGF-II with high affinity (Kₐ between 0.2 and 14.5 nM [see Refs. 6–8]). Presence of the amino-terminal 58 residues of repeat 11 (residues 1508–1566) in a truncated M6P/IGF-II receptor is sufficient for high affinity binding of IGF-II (9). As substitution of isoleucine 1572 by threonine abolishes IGF-II binding (10), the conformation or accessibility of the IGF-II-binding site appears to be sensitive to mutations within repeat 11 that are located outside of the IGF-II-binding site itself.

The M6P-binding sites are critical for the role of the M6P/IGF-II receptor in targeting lysosomal enzymes to lysosomes. Lysosomal enzymes that are synthesized as soluble proteins in the endoplasmic reticulum acquire mannose 6-phosphate residues during their transport through the Golgi, which mediate in the TGN binding to the M6P/IGF-II receptor and/or to a second mannose 6-phosphate receptor of smaller size with an apparent molecular mass of 46 kDa. The receptor-ligand complexes are ferried via AP 1/clathrin-coated vesicles from the TGN to endosomes, where the ligands are released due to an acidic pH and transported to the lysosomes (11). The receptors return back to the TGN or can be transported to the cell surface. Cell surface-associated M6P/IGF-II receptors can bind extracellular ligands and mediate their endocytosis.

IGF-II which binds to M6P/IGF-II receptors at the cell surface is internalized and delivered to lysosomes, where it is degraded (12–14). This is in contrast to the binding of IGF-II to IGF I receptors, which elicits the known metabolic and mitogenic effects of IGF-II through activation of the receptor's tyrosine kinase activity (15). Although not coupled to intracellular signaling cascades, the function of the M6P/IGF-II receptor is critical for the balance between IGF-II, soluble IGF-II binding proteins, and IGF-I receptors. A loss of M6P/IGF-II receptors in mice by targeted disruption leads to an abnormal increase of IGF-II causing fetal overgrowth and perinatal lethality (16). These mice can be rescued by either disrupting the IGF-II or the IGF-I receptor gene (17), suggesting that the perinatal lethality is caused by an excessive activity of the IGF-I receptor stimulated by an excess of IGF-II, which is normally prevented by the M6P/IGF-II mediating the delivery of IGF-II to lysosomes for degradation. The association of hepatocellular carcinomas (18), tumors of the gastrointestinal tract (19, 20), endometrium (21), and breast (22) with a loss of M6P/IGF-II receptor expression has suggested that the M6P/IGF-II receptor acts as a tumor suppressor. Mechanistically, this could be explained by an excess of IGF-II-induced mitogenic stimulation or by a loss of transforming growth factor β-activation (23). The activation of transforming growth factor β, which acts as a tumor suppressor, requires the binding of the latent transforming growth factor β-precursor to the M6P-binding sites of the M6P/IGF-II receptor (24).

To analyze the function of repeat 11 independent of the remaining 14 repeats of the extracellular domain of the M6P/IGF-II receptor, we fused repeat 11 to the transmembrane and cytoplasmic domain of the M6P/IGF-II receptor. The construct was expressed as a stable and IGF-II binding mini-receptor,
which differs in localization from the M6P/IGF-II receptor, but is capable to mediate internalization of IGF-II and its delivery to lysosomes.

EXPERIMENTAL PROCEDURES

Repeat 11 Constructs—Two constructs coding for extracellular repeat 11 of the human M6P/IGF-II receptor were generated. The first construct (HisR11) comprised the single repeat 11(R11) with the signal sequence of preprolactin (nt 60–149). The other construct, R11MT, encoded a membrane-bound protein with the signal sequence of preprolactin at the N-terminus of extracellular repeat 11 (R11) (nt 4669–5094) followed by the transmembrane domain (M) (nt 6991–7128) and cytoplasmic tail (T) (nt 7129–7620) of the human M6P/IGF-II receptor. The constructs were generated by overlap extension-polymerase chain reaction as described (25, 26). In order to generate construct R11MT two overlapping fragments were amplified using the cDNA of wild type M6P/IGF-II receptor as template and the primers SGR 17 (CGGAATTCCGGCAGAAGCTCAATA- AAC) and SGR 22 (AAAGCCCACCGCTTGCTCGCAGGC) and the primers SGR 23 (GCAACGCTGTTGGTGGTACGACG) and SGR 24 (TCCCCCGGTCGACGGTCAATATGTTGAGGATC), respectively. The two fragments served as templates for the external primers SGR 17 and SGR 24. The product was cut with BspEI restriction site, the AGAGGATCGCATCACCAT- CACCATC sequence for the RGS(H6)-tag and the priming sequence that is homologous to the 5′ end of repeat 11 (nt 4669–4687). The 3′ primer was located outside the multicloning site of the expression vector pIC-neo (Promega) with the sequence CACTGCAATCTGACAGTT- GTC. The template AFA for this polymerase chain reaction was re- primer construct 10–11 published previously (9). This polymerase chain reaction construct was cut to give a BspEI/SalI fragment which was inserted into the pC-neo vector containing the signal sequence of preprolactin (nt 60–149).

The other construct, R11MT, encoded a membrane-bound protein using polymerase chain reaction techniques. The 5′ primer included the 5′ BspEI restriction site, the AGAGGATCGCATCACCATC sequence for the RGS(H6)-tag and the priming sequence which differs from the M6P/IGF-II receptor, but is capable to mediate internalization of IGF-II and its delivery to lysosomes.

Expression and Purification of HisR11—BHK cells stably expressing HisR11 were generated as described (27). The amount of R11 protein in the medium was determined by slot blot analysis using a monoclonal antibody against the His epitope (Qiagen, Hilden, Germany). For purification of HisR11, cells were grown for 48 h in 20 ml of DMEM containing 2% fetal calf serum. Medium was collected, centrifuged, and the proteins were precipitated by the addition of 50% (w/v) ammonium sulfate and stored at 4 °C. HisR11 affinity purification using Ni-NTA-agarose matrix (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Eluates from the Ni-NTA-agarose column were further purified using a Superdex-75 gel filtration column (Amersham Pharmacia Biotech) equilibrated with TBS, pH 7.5. The fractions were analyzed by silver staining after SDS-PAGE and by Western blot analysis using an antibody against the His-epitope (28). IGF-II binding was tested by an IGF-II cross-linking assay as described (9).

Surface Plasmon Resonance—The interaction between HisR11 and IGF-II was analyzed in real time by surface plasmon resonance detection (29) using a BIACore 2000 Biosensor (BIACore AB). The purified HisR11 (250 nM) was immobilized to the surface of a NTA sensor chip (carboxymethylated dextran) via its His-tag following the manufacturer's instructions. IGF-II was used at 100 nM in 20 mM NaP, 20 mM NaCl, pH 7.5, and injected at a flow rate of 20 μl/min.

Expression of R11MT—Immortalized mouse embryonic fibroblasts deficient in MPR 46 and M6P/IGF-II receptors (mpr

Degradation of IGF-II—Cells were incubated with 125I-IGF-II for 1 h at 37 °C, chilled to 4 °C, washed three times and incubated for 16 h with 125I-IGF-II (106 cpm/35-mm dish) in the presence or absence of 0.1% saponin. After washing the cells, bound 125I-IGF-II was cross-linked with disuccinimidyl carbonate for 15 min at 4 °C. The reaction was stopped and cells were solubilized and subjected to SDS-PAGE (10% polyacrylamide) under reducing conditions and analyzed by phosphorimaging.

For determination of binding affinity of R11MT toward IGF-II cells were incubated with 125I-IGF-II in the presence of 0.1% saponin and increasing amounts of unlabeled IGF-II (10–10 to 10–14 M). After cross-linking, immunoprecipitation of R11MT and SDS-PAGE (10% polyacrylamide) radioactivity was quantified by phosphorimaging.

Endocytosis of 125I-2C2 Antibody and 125I-IGF-II—Cells expressing R11MT and control cells grown to confluency on 35-mm dishes were incubated with 125I-2C2 antibody (about 200,000 cpm/dish) in 1 ml of DMEM containing 0.1% fetal calf serum for 45 min at 37 °C. Incubation was stopped by rapidly cooling the cells to 4 °C. Medium was collected and cells were washed with ice-cold Hanks’ buffer, followed by washing with 25 mM glycine-HCl, pH 2.5, containing 150 mM NaCl for 2 × 5 min at 4 °C. Cells were harvested in 1 n NaOH and disrupted by ultrasonification. The protein concentration and cell associated radioactivity were determined. For determination of 2C2 bound to the cell surface and resistant to acid wash the cells were incubated for 1.5 h at 4 °C and processed as described above.

To determine endocytosis of 125I-IGF-II, cells grown to confluency on 35-mm dishes were washed twice with phosphate-buffered saline and incubated for 1 h with serum-free DMEM containing 0.1% (w/v) bovine serum albumin. Then the cells were incubated at 37 °C for up to 1 h in 0.6 ml of this medium containing 200,000 cpm of 125I-IGF-II. Incubation was stopped by washing the cells with ice-cold Hanks’ buffer, followed by washing with 0.2 M acetic acid, pH 2.5, containing 0.5 mM NaCl for 2 × 5 min at 4 °C and once more with Hanks’ buffer. The cells were harvested in 1 n NaOH and used for determination of protein and cell associated radioactivity. For determination of unspecific binding, mpr

Degradation of IGF-II—Cells were incubated with 125I-IGF-II for 1 h at 37 °C as described above. The cells were then washed with 10 mM phosphate buffer, pH 4.5, containing 2.54 mM CaCl2, 0.9% NaCl, and 5 mg/ml bovine serum albumin. Cells were incubated for 5 h at 37 °C in DMEM with 10% fetal calf serum. After the chase the medium was collected, and radioactivity in the medium, the washing fraction, and in the cells was determined. The trichloroacetic acid-soluble radioactivity released to the medium during the chase was determined by precipitation with 0.2 mg/ml trichloroacetic acid on ice for 2 h, followed by centrifugation and counting of the supernatant and precipitate.
A Recombinant Mini-receptor for IGF-II

RESULTS

Repeat 11 Is Sufficient to Bind IGF-II—Repeat 11 (residues 1508–1650) was expressed in baby hamster kidney cells as a fusion protein containing the signal sequence of preprolactin at the NH₂ terminus followed by RGSH₆ and repeat 11 of the human M6P/IGF-II receptor (HisR11 in Fig. 1). Clones secreting about 1 µg of HisR11/ml medium in 24 h were isolated. HisR11 protein was purified to homogeneity by Ni-NTA-agarose. The apparent molecular mass of 18 kDa corresponds to the calculated weight of the unglycosylated HisR11 protein (Fig. 2A). Amino-terminal sequencing confirmed the integrity of the NH₂ terminus of HisR11 and revealed that the only potential N-glycosylation site (position 1520 of the wild-type receptor) is not glycosylated (data not shown). In cross-linking experiments we analyzed binding of IGF-II in the presence of various amounts of unlabelled IGF-II (Fig. 2B) and determined a half-maximal inhibition constant (IC₅₀) of 0.1 nm. To determine the equilibrium rate constant (Kᵣ) of the interaction between IGF-II and HisR11 we coupled HisR11 to a sensor chip for analysis by surface plasmon resonance. IGF-II bound to coupled HisR11 with a Kᵣ of 23 nm, a 2-fold lower affinity when compared with the IC₅₀ of 10 nm that was found by the cross-linking experiments. Taken together, these data show that repeat 11 is sufficient for high affinity binding of IGF-II.

Expression and Localization of Repeat 11 Fused to the Transmembrane Domain and Cytoplasmic Tail of M6P/IGF-II Receptor—The role of the M6P/IGF-II receptor in the physiology of the IGF-II system is considered to the control of the concentration of IGF-II in tissues and body fluids by delivering IGF-II through receptor-mediated endocytosis to lysosomes, where IGF-II is degraded. To analyze whether repeat 11 is sufficient to trigger endocytosis of IGF-II and its transport to lysosomes we constructed a type I membrane protein with the extracytoplasmic domain represented by R11 fused to the transmembrane domain (M) and cytoplasmic tail (T) of the human M6P/IGF-II receptor (R11MT). Both constructs contain the cleavable signal sequence of preprolactin (S) at the NH₂ terminus, HisR11 additionally a RGSH₆-tag (H). The NH₂-terminal and COOH-terminal residues of the receptor domains are indicated (for numbering see Ref. 52).

The intracellular localization of R11MT was compared with that of wild-type M6P/IGF-II receptor expressed in mpR cells by indirect immunofluorescence using an antibody directed against the cytoplasmic tail of M6P/IGF-II receptor. R11MT was localized in punctate structures distributed throughout the cytoplasm (Fig. 4B), while the wild-type receptor was also concentrated in perinuclear structures (Fig. 4A). Colocalization with γ-adaptin, a commonly used TGN marker (42, 43), demonstrated that the perinuclear structures mainly represent TGN (Fig. 4C). On the other hand, little if any R11MT colocalized with γ-adaptin (Fig. 4D). Partial colocalization was observed for R11MT and the transferrin receptor, a marker for recycling endosomes and presumably other endosomal subcompartments (44). This colocalization was much more prominent for the R11MT than for the wild-type M6P/IGF-II receptor (compare Fig. 4, F and E). Neither R11MT nor the wild-type M6P/IGF-II receptor colocalized with the late endosomal/lysosomal markers lamp1 (Fig. 4, H and G) and lyso-lysosphatidic acid (not shown).

To determine the distribution of R11MT between the plasma membrane and intracellular membranes we used the monoclonal antibody 2C2 raised against the human M6P/IGF-II receptor. This antibody inhibits binding of IGF-II to the M6P/IGF-II receptor (37) and is therefore likely to recognize repeat 11. Cells expressing R11MT or the intact M6P/IGF-II receptor were incubated with ¹²⁵I-2C2 at 4 °C in the absence or presence of saponin. In the absence of saponin the antibody can only bind to cell surface exposed receptors, while in cells permeabilized
with saponin it binds also to receptors in intracellular membranes. In permeabilized cells expressing R11MT the amount of bound $^{125}$I-IGF-II was 4–5-fold higher than in cells expressing wild-type receptor. Binding to non-transfected cells was up to 2% of that to R11MT expressing cells (Fig. 5). The fraction of R11MT expressed at the cell surface was 24%. This was about 2.5-fold higher than observed for the wild-type receptor (9%, Fig. 5).

These results were confirmed by cross-linking $^{125}$I-IGF-II to R11MT (Fig. 6). After incubation of cells for 16 h at 4 °C with $^{125}$I-IGF-II in presence or absence of saponin, unbound $^{125}$I-IGF-II was removed by washing and cells were exposed to the cross-linker disuccinimidyl suberate. Cells were homogenized and subjected to SDS-PAGE. Phosphorimaging of the gels revealed a signal with an apparent molecular mass of 46 kDa, which corresponds to the molecular mass expected for the complex of R11MT (39 kDa) with IGF-II (7.5 kDa). In agreement with the values obtained from labeling with $^{125}$I-2C2 antibody, 24% of total bound $^{125}$I-IGF-II were found at the surface of cells expressing R11MT. In the presence of $10^{-6}$ M unlabeled IGF-II binding of $^{125}$I-IGF-II was reduced to 26% indicating the specificity of binding IGF-II. Taken together, these results show that the intracellular distribution of the IGF-II mini-receptor differs from that of the wild-type receptor. The mini-receptor could not be detected at the TGN but in endosomal compartments missing the wild-type receptor. At steady state the fraction at the cell surface is about 2.5-fold higher than observed for wild-type receptor. It is notable that for the wild-type receptor the expression level never exceeded that of endogenous M6P/IGF-II receptor more than 2–3-fold (31), while significantly higher expression levels were tolerated for R11MT.

**Stability of R11MT and Affinity to IGF-II**—Stability of R11MT was determined by metabolic labeling cells with $^{[35]}$S-methionine for 1 h followed by a chase for up to 24 h. $^{[35]}$SIR11MT was immunoprecipitated, separated by SDS-PAGE, and quantified by phosphorimaging (Fig. 7). The appar-

![Fig. 4. Intracellular distribution of R11MT.](image)

**Fig. 4.** Intracellular distribution of R11MT. mpr$^-$/cells expressing either R11MT (B, D, F, and H) or M6P/IGF-II receptor (A, C, E, and G) were fixed, permeabilized with Triton X-100, and labeled with an antibody against the cytoplasmic domain of the M6P/IGF-II receptor (A and B) and double-labeled with antibodies against $\gamma$-adaptin (C and D), transferrin receptor (E and F), and lamp 1 (G and H). Bound antibodies were detected using fluorochrome-labeled antibodies causing green (C and D, $\gamma$-adaptin; E-H, IGF-II receptors) and red (C and D, IGF-II receptors; E-H, transferrin receptor and lamp 1) fluorescence as described under “Experimental Procedures.” The fluorograms in A, C and B, D, respectively, show identical cells.

![Fig. 5. Binding of antibody 2C2 to R11MT and wild-type receptor.](image)

**Fig. 5.** Binding of antibody 2C2 to R11MT and wild-type receptor. mpr$^-$/cells expressing either R11MT, wild-type receptor and non-transfected control cells were incubated for 1.5 h at 4 °C with $^{125}$I-iodinated antibody 2C2 in the presence or absence of saponin. Afterward, cells were washed, harvested, and subjected to $\gamma$-counting. Binding to intracellular and cell surface-associated receptors (binding in the presence of saponin) is represented by the bars. The fraction of radioactivity bound to cell surface (binding in the absence of saponin) is represented by the gray of the bars. The values represent the mean of four to seven experiments and the standard deviation.

![Fig. 6. Binding of IGF-II to R11MT at the cell surface and intracellular membranes of mpr$^-$/cells.](image)

**Fig. 6.** Binding of IGF-II to R11MT at the cell surface and intracellular membranes of mpr$^-$/cells. Cells expressing R11MT and control cells were incubated with $^{125}$I-IGF-II for 16 h at 4 °C in the presence or absence of saponin and $10^{-6}$ M unlabeled IGF-II as indicated. Cells were washed and cell-bound IGF-II was cross-linked to the cells with disuccinimidyl suberate. Afterward, cells were harvested and subjected to SDS-PAGE. Radioactivity in position of the cross-linked IGF-II receptor complexes (arrowhead) was quantified by phosphorimaging. The apparent molecular weight of a standard protein is indicated in kDa.
ent half-life of R11MT turned out to be 10 h, comparable to that of the intact M6P/IGF-II receptor (10–29 h, Refs. 45–48).

In order to determine the affinity of IGF-II binding unlabeled IGF-II was added in increasing concentrations during incubation of cells for 16 h at 4 °C in the presence of 125I-IGF-II and saponin. After cross-linking, immunoprecipitation of R11MT, and SDS-PAGE, radioactivity associated with the complex of R11MT and IGF-II was quantified (Fig. 8). Binding of 125I-IGF-II was inhibited to 50% in the presence of 100 nM unlabeled IGF-II. This result indicates that IGF-II binds to R11MT with 10-fold lower affinity than to the soluble repeat 11 (see Fig. 2).

Endocytosis of 2C2 Antibody and IGF-II by R11MT—A receptor responsible for down-regulation of extracellular IGF-II should mediate the internalization of IGF-II bound at the cell surface and its delivery to lysosomes. To test for the internalization of cell surface-associated R11MT we incubated cells expressing R11MT for 1–3 h at 37 °C in the presence of 125I-2C2 antibody. Surface-bound radioactivity was removed by an acid wash and the remaining intracellular radioactivity was determined (Fig. 9). 125I-2C2 antibody was rapidly internalized by R11MT expressing cells, whereas in untransfected mpr cells or in cells incubated at 4 °C, the internalization was below the level of detection (Fig. 9). In the medium the decrease of radioactivity paralleled the uptake of radioactivity by the cells (not shown). The amount of trichloroacetic acid-soluble radioactivity in the medium remained constant (about 5% of total), indicating that degradation of internalized 2C2 was negligible. These data indicate that 2C2 antibody bound by R11MT at the cell surface is rapidly endocytosed, but not degraded within 3 h.

Endocytosis of IGF-II was tested by incubating cells in the presence of 125I-IGF-II. Nonspecifically bound IGF-II (as defined as the fraction of IGF-II bound to mpr cells kept on ice) was difficult to remove. Even after washing mpr cells with 0.2 m acetic acid and 0.5 m NaCl, the amount of radioactivity associated with mpr cells was almost two-thirds of that associated with R11MT expressing cells when incubated at 4 °C (see Fig. 10, open symbols). Nevertheless, when the cells were incubated at 37 °C for up to 1 h in the presence of 125I-IGF-II, a time dependent association of IGF-II with R11MT expressing cells was noted, while the amount of IGF-II associated with mpr cells remained constant with time (Fig. 10). Presence of 1 μM unlabeled IGF-II decreased the R11MT-dependent association of 125I-IGF-II with cells by 80% (Fig. 10). The amount of
IGF-II that associated within 1 h at 37 °C with R11MT and wild-type M6P/IGF-II receptor expressing cells was comparable and about 3-fold higher than with mpR cells (Table I).

In order to follow the degradation of the cell associated 125I-IGF-II, we chased the cells with unlabeled medium for 5 h following the endocytosis period. However, washing of the cells after endocytosis and prior to the chase period had to be done more gently on the expense of a higher background. After chase the medium was assayed for trichloroacetic acid-soluble radioactivity. Radioactivity released by the cells expressing R11MT and wild-type M6P/IGF-II receptor corresponded to 24 and 58%, respectively, of the radioactivity associated with the cell medium after the chase for 5 h. Trichloroacetic acid-soluble radioactivity in the medium was determined.

At steady state the IGF-II mini-receptor is barely detectable in perinuclear structures, one of the predominant localization of the wild-type receptor. These perinuclear structures are positive for the clathrin-associated adaptor complex AP1 and therefore represent the TGN. Some of these structures, however, may also represent endosomal structures, since recent reports indicate that the M6P/IGF-II receptor is transported back from endosomes to the Golgi via AP 1/clathrin-coated vesicles (50). Trafficking signals necessary for sorting of newly synthesized lysosomal enzymes and for internalization of ligands have been localized to the cytoplasmic domain of the M6P/IGF-II receptor. While the IGF-II mini-receptor cycles between endosomes and the cell surface, the present data do not allow to answer the question whether the mini-receptor is also capable of cycling repeatedly between endosomes and the Golgi where the steady state concentration is much lower than that of the M6P/IGF-II receptor. This indicates that the extra- and cytoplasmic domain of the M6P/IGF-II receptor strongly influences the trafficking behavior of the receptor. An influence of the extracytoplasmic domain of the M6P/IGF-II receptor on its trafficking was already noted earlier, e.g., a fusion protein, in which the extracytoplasmic domain of the M6P/IGF-II receptor was replaced by that of the epidermal growth factor receptor, was unable to be transported from the cell surface to the TGN (51).

An additional difference between the IGF-II mini-receptor and wild-type receptor was noted for the efficiency with which internalized IGF-II is degraded. While the lower degradation efficiency observed in cells expressing the IGF-II mini-receptor may well point to a different routing of the IGF-II mini-receptor and its ligand within the endosomal system, we consider another explanation to be more likely. The cells in which the receptors were expressed lack endogenous mannose 6-phos-
phate receptors. Therefore, they missort newly synthesized lysosomal enzymes into the secretions and display a severe deficiency of lysosomal enzymes including proteinases in their lysosomes. Expression of the wild-type M6P/IGF-II receptor reverts this multiple lysosomal enzyme deficiency (31). This lysobisphosphatidic acid.

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