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# Insulin-like growth factor-II/cation-independent mannose 6-phosphate receptor in neurodegenerative diseases

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## Abstract

The insulin-like growth factor II/mannose 6-phosphate (IGF-II/M6P) receptor is a multifunctional single-transmembrane glycoprotein. Recent studies have advanced our understanding of the structure, ligand binding properties and trafficking of the IGF-II/M6P receptor. This receptor has been implicated in a variety of important cellular processes including growth and development, clearance of IGF-II, proteolytic activation of enzymes and growth factor precursors, in addition to its well-known role in the delivery of lysosomal enzymes. The IGF-II/M6P receptor, distributed widely in the central nervous system, has additional roles in mediating neurotransmitter release and memory enhancement/consolidation, possibly through activating IGF-II-related intracellular signaling pathways. Recent studies suggest that overexpression of the IGF-II/M6P receptor may have an important role in regulating the levels of transcripts and proteins involved in the development of Alzheimer's disease (AD) – the prevalent cause of dementia affecting the elderly population in our society. It is reported that IGF-II/M6P receptor overexpression can increase the levels/processing of amyloid precursor protein leading to the generation of β-amyloid peptide, which is associated with degeneration of neurons and subsequent development of AD pathology. Given the significance of the receptor in mediating the transport and functioning of the lysosomal enzymes, it is being considered for therapeutic delivery of enzymes to the lysosomes to treat lysosomal storage disorders. Notwithstanding these results, additional studies are required to validate and fully characterize the function of the IGF-II/M6P receptor in the normal brain and its involvement in various neurodegenerative disorders including AD. It is also critical to understand the interaction between the IGF-II/M6P receptor and lysosomal enzymes in neurodegenerative processes, which may shed some light on developing approaches to detect and prevent neurodegeneration through the dysfunction of the receptor and the endosomal-lysosomal system.

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**Keywords**

Insulin-like growth factor II receptor; Mannose 6-phosphate receptor; Endosomal–lysosomal system; Alzheimer's disease; Neurodegenerative diseases

## 1. Introduction

Insulin-like growth factors I and II (IGF-I and IGF-II) are pleiotropic polypeptides that are distributed widely in various tissues/organs, including the central nervous system (CNS). They share structural homology with proinsulin and are considered to mediate a wide spectrum of physiological functions during development and in the adult. At the cellular level, both IGFs can act as autocrine and paracrine factors to regulate cellular growth, survival, differentiation and chemotaxis. As components of an endocrine system, IGFs also help to regulate general growth and metabolism [1, 2]. The biological activities of IGF-I and -II are regulated by their synthetic rates, clearance, and their binding to a family of high-affinity IGF binding proteins (IGFBP1-6) and low-affinity IGFBP-related peptides (IGFBP-rP1-4) [3]. These proteins influence the half-life of circulating and tissue IGFs by regulating their transport in the circulation, filtration and excretion by the kidneys, delivery to their target cells, and interaction with receptors [4]. Generally, binding to IGFBPs decreases the activity of IGFs while nevertheless decreasing clearance and extending their biological half-life. The evidence further suggests that IGF stimulation increases the synthetic rates of some binding proteins, thus providing a feedback regulation in controlling the activity of the growth factors at the tissue level. Adding to the complexity of IGF availability, several enzymes capable of proteolyzing IGFBPs have been identified. The cleavage of IGFBPs by IGFBP proteases plays a key role in modulating the levels of free IGFs and IGFBPs and their actions [5, 6].

The biological functions of both IGFs are mediated by specific membrane receptors referred to as the IGF-I, IGF-II and insulin receptors (Fig. 1) [1, 7]. The IGF-I receptor is a member of the tyrosine kinase receptor family with close structural homology to the insulin receptor. This receptor binds IGF-I with higher affinity than either IGF-II or insulin and is usually located at the cell surface as a heterotetramer consisting of two α (135 kDa) and two β (90 kDa) subunits held together by disulfide bonds. The α-subunits contain the extracellular ligand binding site, whereas the β-subunits have transmembrane and tyrosine kinase domains and an intracellular tyrosine autophosphorylation site [7]. Ligand binding to the extracellular α subunit induces a conformational change that triggers receptor tyrosine kinase activity and initiates autophosphorylation of tyrosine residues within the intracellular segment of the β subunit (transphosphorylation) [1, 7]. This event leads to the docking of effector and adaptor molecules and subsequent activation of various intracellular signaling cascades, including the mitogen-activated protein (MAP) kinase and phosphoinositide 3′-kinase pathways, which regulate growth, proliferation, survival, development and metabolic responses [1, 7]. Although the IGF-I receptor is the primary mediator of IGF's physiological effects, the insulin receptor (IR), which binds both insulin and the IGFs, can also mediate certain biological actions of IGF-I and -II [7]. The IR is expressed as two isoforms, IR-A and IR-B, due to alternative splicing of exon 11 - a small exon encoding 12 amino acid

residues at the carboxyl terminus of the IR α-subunit. Some studies have indicated that IGF-II binds IR-A, the isoform that excludes the exon-11 peptide, with higher affinity than IR-B in a variety of tissues and malignant cells [8, 9]. Activation of IR-A by IGF-II has been shown to stimulate mitogenic effects in IGF-I receptor-null mouse embryonic fibroblasts, possibly *via* the coordinated activation or deactivation of the proto-oncogenic serine kinase, Akt, glycogen synthase kinase 3-β, and extracellular-signal-regulated kinases (ERKs) [10]. The role of IR-B in mediating IGF effects remains unclear. Apart from specific IGF-I and insulin receptors, the detection of a hybrid receptor, comprising an insulin receptor αβ hemimolecule and an IGF-I receptor αβ hemimolecule, has added an additional layer of complexity to the IGF system. Although the hybrid receptors are widely distributed in certain tissues [11, 12], their signaling characteristics and/or physiological relevance in relation to the IGF-I receptor are still under investigation. Nevertheless, receptor cross-talk interactions through shared ligands, subunit combination and phosphorylation of some common substrates, are believed to be the main mechanisms by which both IGFs can mediate their functions [1, 2, 7].

The IGF-II receptor, unlike the IGF-I or insulin receptors, is structurally distinct and has no intrinsic tyrosine kinase activity. It exhibits higher affinity for IGF-II than IGF-I and does not bind insulin (Fig. 1) [13, 14]. The discovery in 1987–88 [15–17] that the IGF-II receptor is identical to the cation-independent mannose 6-phosphate (M6P) receptor indicates that this receptor (i.e., the IGF-II/M6P receptor) could function in multiple biological processes. Indeed, several studies have clearly established a role for this receptor in lysosomal enzyme trafficking from the trans-Golgi network (TGN) to the endosome for their subsequent delivery to lysosomes, clearance and/or activation of a variety of growth factors and endocytosis-mediated degradation of IGF-II. A growing body of evidence further supports a role for this receptor in transmembrane signal transduction in response to IGF-II binding, but its biological relevance remains controversial. At present, unlike the IGF-I receptor, very little is known as to the physiological and/or pathological significance of the IGF-II/M6P receptor in the functioning of the CNS [13, 18]. Thus, there is a need for a better understanding of the potential implications of this unique multifunctional receptor. This review provides an overview of the current knowledge on the structure and function of the IGF-II/M6P receptor, with special emphasis on its role in CNS functions in the normal brain and in degenerative diseases. For information on earlier work on the IGF-II/M6P receptor and/or its role in the periphery, the reader is referred to several reviews [13, 18, 19].

## 2. Structure, ligands and trafficking of the IGF-II/M6P receptor

### 2.1. Primary structure of the IGF-II/M6P receptor

Structurally, the IGF-II/M6P receptor is a type 1 integral membrane glycoprotein and P-type lectin receptor with a large N-terminal extracytoplasmic domain (ectodomain), a single transmembrane domain and a short C-terminal cytoplasmic domain. The ectodomain, which protrudes into the extracellular space or the lumen of vesicles and intracellular organelles, is composed of 15 contiguous repeats of approximately 147 amino acid residues each, sharing 14–38% sequence identity. Most repeats contain eight conserved cysteines that form intramolecular disulfide bonds necessary for proper receptor folding [13, 20, 21].

Functionally, the receptor binds IGF-II and M6P-containing ligands at distinct sites: repeat 11 comprises the core IGF-II binding site, whereas repeats 3, 5, 9 and 15 bind substrates with M6P groups [20, 22–24] (Fig. 1). High-resolution crystal structure studies of repeat 11 and of a receptor fragment consisting of repeats 1–3 of the human IGF-II/M6P receptor have provided insights into the structural features of the receptor [20, 21]. These studies suggest that all 15 repeats share a similar topology consisting of a flattened β-barrel formed by nine β-strands. This overall disulfide bond-stabilized structure has been termed a mannose 6-phosphate receptor homology (MRH) domain [13]. Further analysis of the 1–3 triple-repeat crystal indicates a structure in which repeat 3 sits on the top of repeats 1 and 2, suggesting that the IGF-II/M6P receptor forms distinct structural units that stack in a back-to-front manner. In this model, the IGF-II binding site potentially resides on the opposite face of the structure relative to the principal M6P binding sites of domains 3 and 9. The ectodomain contains 19 potential glycosylation sites, of which at least two are utilized in forming the mature receptor. Post-translational modifications including phosphorylation and palmitoylation have also been reported for the receptor. The cytoplasmic domain contains motifs that are important for receptor trafficking and phosphorylation. For example, the single tyrosine-based internalization motif, YSKV, is involved in targeting plasma membrane receptors to clathrin-coated vesicles [25]. Additionally, several regions of the cytoplasmic tail can act as potential substrates for various protein kinases, including protein kinase C (PKC), cAMP-dependent protein kinase, and casein kinases I and II [26]. A truncated form of the receptor representing a proteolytic cleavage product of the receptor's ectodomain has been identified in serum and other physiological fluids of a variety of mammalian species [27, 28]. The significance of the truncated receptor form in relation to the functioning of the IGF-II/M6P receptor under *in vivo* conditions remains unclear; however, it appears that proteolytic cleavage at the cell surface to release the receptor's ectodomain may be one means to degrade and thus down-regulate levels of the IGF-II/M6P receptor [29, 30].

The human IGF-II/M6P receptor gene is located on chromosome 6 and the murine IGF-II/M6P receptor gene is located on chromosome 17, and both contain 48 exons [31]. Interestingly, the exon boundaries of the IGF-II/M6P receptor, unlike other multidomain receptors, do not correspond to its functional or structural domains: exons 1–46 encode the receptor's ectodomain with each of its 15 domains encoded by portions of 3 to 5 separate exons. In the human, expression of the IGF-II/M6P receptor gene is biallelic, whereas in the mouse it is maternally imprinted in peripheral tissues [32] but is expressed from both parental alleles in the CNS. The IGF-II/M6P receptor is ubiquitously expressed in most cells and tissues, and a number of studies have demonstrated that levels of the receptor are developmentally regulated; expressed at higher levels during fetal development and then declining over the postnatal period [33–35].

### 2.2. Ligands of the IGF-II/M6P receptor

As stated above, the multifunctional IGF-II/M6P receptor binds M6P-containing ligands and IGF-II at several distinct sites (Table 1) [13, 22, 24]. Two high-affinity M6P binding sites localize to repeats 1–3 and 7–11 of the receptor's ectodomain, with essential residues localized to domains 3 and 9 (Fig. 1). A third, lower-affinity M6P recognition site that has a

preference for binding mannose 6-phosphodiesters has been demonstrated within domain 5 [23]. Recently, a fourth M6P binding site with very low ligand-binding affinity has been localized to domain 15 [24]. It has been demonstrated that one receptor can simultaneously bind two molecules of M6P and one molecule of IGF-II [13, 36]. A variety of M6P-containing ligands such as lysosomal enzymes, transforming growth factor-β (TGF-β) [37], granzyme B, glycosylated leukemia inhibitory factor (LIF) [38], proliferin [39] and thyroglobulin [40], Other ligands that may bind the receptor by a non-M6P-based interaction are retinoic acid [41], urokinase-type plasminogen activator receptor (uPAR) and plasminogen itself [42, 43]. It is noteworthy that of the receptor's 15 ectodomain repeats, ligand-binding functions - either primary (1, 3, 5, 9, 11, 15) or secondary (1, 7, 13) - have been ascribed to every odd-numbered repeat, whereas the even-numbered domains are considered to have more passive functions involving connection, spacing, orientation or dimerization.

The distinct binding sites of the IGF-II/M6P receptor allow not only for simultaneous binding of IGF-II and M6P-tagged glycoproteins, but binding of one ligand has been shown to reciprocally modulate receptor affinity for other ligands [44, 45]. For example, IGF-II has been shown to prevent binding of β-galactosidase to purified IGF-II/M6P receptors, whereas several lysosomal enzymes, but not M6P, inhibit the binding of IGF-II to the receptor [46, 47]. Conversely, M6P has been shown to stimulate the binding of $^{125}$I-IGF-II to the IGF-II/M6P receptor by two-fold in a number of cell types [45, 48]. Although the physiological significance of this interaction remains to be defined, it has been suggested that reciprocal inhibition of binding of these two classes of ligand is probably caused by a steric hindrance or a conformational change in the receptor. As a consequence of this reciprocal inhibition, extracellular lysosomal enzymes may inhibit the IGF-II/M6P receptor-mediated degradation of IGF-II, whereas the presence of IGF-II would increase the concentration of extracellular lysosomal enzymes. This issue will be discussed in further detail below.

Traditionally thought to function as a monomer, the IGF-II/M6P receptor exists in the membrane as an oligomer, and simultaneous, cooperative binding by monomers is necessary to produce a high affinity for M6P-related ligands [49, 50]. The ectodomain repeat 12 is believed to be functionally important for dimerization of the receptor (Fig. 1), which apparently enhances the binding affinity of ligands that are multivalent for M6P residues and alters the kinetics of receptor internalization from the cell surface [49, 50]. As IGF-II and lysosomal enzymes act as primary ligands for the multifunctional IGF-II/M6P receptor, the following section provides a brief overview of the features that underlie the binding of these ligands to the receptor.

**2.2.1. IGF-II—**IGF-II, which exhibits close structural similarity with IGF-I and insulin, contains 67 amino acid residues of which 45 (62%) are identical to IGF-I [51]. The gene encoding IGF-II maps to chromosome 11p15 and comprises 9 exons and 4 promoters. Exons 7, 8 and 9 encode the prepro-IGF-II protein, whereas exons 1 to 6 are non-coding and form alternative 5′-untranslated regions [52]. The 180-residue prepro-IGF-II contains an 89-residue carboxyl-terminal (E) peptide and a 24-residue signal peptide, both of which are cleaved post-translationally to generate mature IGF-II. At the cellular level, IGF-II and its mRNA are widely distributed in many different tissues including the CNS. Although IGF-II

binds to both the IGF-I and IR-A receptors, it is considered to be the best-characterized non-M6P-containing ligand of the IGF-II/M6P receptor in viviparous mammals [13]. Functionally, IGF-II binds to repeat 11 of the IGF-II/M6P ectodomain [53, 54], which contains two hydrophobic binding sites, the first being a shallow cleft located at the mouth of the β-barrel and the second one extends along an external flattened surface. The former binding site, which contains $Ile^{1572}$, is crucial for the initial docking of IGF-II, whereas the latter plays a role in stabilizing IGF-II binding [55, 56]. Structural studies of the interactions between IGF-II and the receptor, confirmed by mutagenesis, demonstrate that the residues $Phe^{19}$ and $Leu^{53}$ of IGF-II lock into this hydrophobic pocket of the receptor [57]. Repeat 11 of the ectodomain is also sufficient to mediate internalization of IGF-II by the receptor [56]. Examination of IGF-II binding to mini-receptors containing repeats 11–12, 11–13, and 11–15 indicates that repeat 13 does not itself bind to IGF-II but contains a domain that enhances its binding to the receptor [18, 58, 59]. It is also of interest to note that IGF-II binding by the IGF-II/M6P receptor is mostly confined to viviparous mammals, as platypus [60], chicken [61] and frog [61] receptors do not demonstrate such interactions or they bind with such low affinity as to not be physiologically relevant. The sequence alignment studies of both the IGF-II/M6P receptor and IGF-II suggested that although IGF-II remains relatively unaltered during evolution, the receptor gained the ability to bind IGF-II by changing key residues located in the binding pocket. Interestingly, unlike IGF-II binding, the carbohydrate recognition function of the receptor is widely utilized by mammalian and non-mammalian species [62, 63].

**2.2.2. Lysosomal enzymes—**To date, approximately 60 lysosomal hydrolases have been identified [64], and the majority of them are transported to lysosomes *via* the IGF-II/M6P receptor [62]. Equilibrium dialysis experiments have shown that the receptor can bind 2 moles of M6P or 1 mole of β-galactosidase or equivalent lysosomal enzyme bearing multiple M6P moieties [13]. Site-directed mutagenesis studies combined with pentamannosyl phosphate-agarose chromatography and binding affinity analyses have identified 5 amino acids in both repeat 3 (Q392, S431, R435, E460 and Y465) and repeat 9 (Q1292, H1329, R1334, E1354 and Y1360) that are essential for carbohydrate recognition by the bovine IGF-II/M6P receptor [13, 65]. Structure-based sequence alignment analysis of repeat 5 has also revealed 4 key residues (Gln, Arg, Glu and Tyr) that are necessary for carbohydrate binding, but the affinity of this site for M6P is approximately 300-fold lower than those of repeats 3 and 9 [23]; this site has now been identified as having a preference for binding mannose 6-phosphodiesters [66]. The C-terminal M6P binding site located on domain 9 exhibits optimal binding at pH 6.4–6.5, whereas the N-terminal M6P binding site of domain 3 shows a higher optimal binding pH of 6.9–7.0. Furthermore, the C-terminal site is highly specific for M6P and M6P phosphomonoester, whereas the N-terminal site binds M6P phosphodiester and M6P-sulfate with lower affinity than M6P [13, 67]. A recent study revealed that domain 15 has 3 of the five canonical ligand-binding residues and does bind M6P ligands, but with very low affinity [24, 66]; the physiological relevance of this property is unclear at this time. Thus, it is apparent that carbohydrate-binding sites of the IGF-II/M6P receptor recognize a great diversity of ligands over a relatively broad pH range [68].

### 2.3. Trafficking of the IGF-II/M6P receptor

Under normal conditions, the IGF-II/M6P receptors are localized mostly in the TGN and endosomal compartments and to a lesser extent (~10%) on the plasma membrane, but the receptors continuously shuttle between the surface and intracellular pools [18, 59]. Several agents, including growth factors, enzymes and chemical compounds, have been shown to modulate cellular recycling and routing of the IGF-II/M6P receptor. For example, a rapid and transient redistribution of IGF-II/M6P receptors from internal pools to the cell surface is induced in human fibroblasts by IGF-I, IGF-II and epidermal growth factor (EGF) [69]. The most striking effects on the distribution of the IGF-II/M6P receptor have been observed in rat adipocytes and H-35 hepatoma cells, wherein insulin causes a major subcellular relocalization of receptors from internal membranes to the cell surface [70, 71]. Glucose also increases IGF-II binding to the IGF-II/M6P receptor as a result of increased receptor cell-surface localization in two insulin-secreting cell lines (RINm5F and HIT) and human erythroleukemia K562 cell line [72]. Furthermore, the lysosomal enzyme β-glucuronidase has been shown to increase the rate of receptor internalization from the cell surface by stimulating or stabilizing receptor dimerization [73], whereas some major histocompatibility complex class I-derived peptides have been shown to inhibit receptor internalization in insulin-stimulated rat adipose cells [74]. Although the underlying mechanism(s) remains to be established, several kinases and phosphatases have been suggested to regulate the redistribution of cellular IGF-II/M6P receptors [19]. There is evidence that PKC-mediated serine phosphorylation or okadaic acid inhibition of serine phosphatases increases the proportion of receptors on the plasma membrane [72, 75, 76]. The cytoplasmic domain of the bovine IGF-II/M6P receptor contains three serine residues (i.e., $Ser^{19}$, $Ser^{85}$ and $Ser^{156}$) whose phosphorylation correlates with the localization of the receptor in the TGN and clathrin-coated vesicles [77–79]. However, disruption of these three phosphorylation sites by mutagenesis had no detectable effect on the sorting of lysosomal enzymes by the bovine or murine IGF-II/M6P receptor [25].

## 3. Functions of the IGF-II/M6P receptor

The IGF-II/M6P receptor is actively involved in the regulation of cellular homeostasis either by transporting a diverse group of extracellular ligands into the cell *via* clathrin-coated vesicles for their subsequent activation/degradation or by targeting molecules such as lysosomal enzymes to endosomes/lysosomes for their subsequent functions. Binding of IGF-II to the receptor located on the plasma membrane may also exert some biological effects under certain circumstances by activating specific signal transduction pathways. Although the precise function of the IGF-II/M6P receptor may vary depending on the ligands and/or cell type under consideration, here we provide a brief overview of the general functions of IGF-II/M6P receptors that have been studied rather extensively over the years [26].

### 3.1. IGF-II/M6P receptor in sorting of lysosomal enzymes

Although the segregation and transport of lysosomal enzymes are believed to be mediated by a number of selective receptors, several lines of evidence demonstrate a critical role for IGF-II/M6P receptor in intracellular sorting of newly synthesized lysosomal enzymes. The lysosomal enzymes, after being synthesized in the rough endoplasmic reticulum (ER), enter

the lumen of the ER by means of an amino-terminal signal peptide. Co-translational glycosylation occurs on selected asparagine residues by transfer of pre-formed oligosaccharides rich in mannose residues. The lysosomal enzymes are then moved by vesicular transport to the Golgi stack, where phosphorylation of selected mannose residues on the 6-position is performed in a two-step process. First, a phosphotransferase transfers N-acetylglucosamine-1-phosphate from UDP-GlcNAc to a mannose residue on the lysosomal enzyme, resulting in a phosphodiester intermediate. Subsequently, N-acetylglucosamine-1-phosphodiester α-N-acetylglucosaminidase removes N-acetylglucosamine, yielding a mannose 6-phosphomonoester that can bind to the receptor. As the lysosomal enzymes acquire their complement of M6P residues in the cis-Golgi, they become capable of binding to the IGF-II/M6P receptors that are also being directed through the Golgi stacks. Sorting of lysosomal enzymes away from other transmembrane and secreted protein cargo usually occurs in the TGN, where clathrin-coated vesicles are found to arise from the tubular structures. The vesicles containing lysosomal enzymes are then trafficked to late endosomes, where the acidic milieu triggers the dissociation of lysosomal enzymes from the IGF-II/M6P receptor. While the lysosomal enzymes are subsequently delivered to lysosomes by vesicular trafficking, the unoccupied IGF-II/M6P receptors are either targeted to the cell surface or recycled to the TGN to engage in another round of transport of ligands (Fig. 2) [13, 26]. A minor population of lysosomal enzymes escapes the intracellular sorting and gets secreted through the constitutive secretory trafficking pathway; these molecules bind to cell-surface IGF-II/M6P receptors and are transported to the lysosomes *via* clathrin-coated endocytic vesicles. The endocytic pathway that leads to delivery of the enzymes to lysosomes utilizes the same late-endosome/pre-lysosomal intermediates that are utilized in the intracellular biosynthetic pathway [80]. Recent evidence suggests that IGF-II/M6P receptor can also mediate transcytosis of lysosomal enzymes across the blood-brain barrier (BBB) in neonatal mice [81], thus providing an underlying basis for the treatment of lysosomal storage disorders characterized by neurological impairment arising from deficits in specific lysosomal enzymes [82].

Site-directed mutagenesis experiments have shown that binding of clathrin-associated proteins to an acidic-cluster-dileucine amino acid (AC-LL) motif within the cytoplasmic tail of the M6P receptors is required for efficient clathrin-mediated transport of lysosomal enzymes to endosomal compartments [18, 83]. Previously, interactions between the clathrin adaptor protein AP1 and the dileucine-based sorting signals of M6P receptors, in conjunction with ADP-ribosylation factor, were thought to mediate clathrin-coat assembly on vesicles budding from the TGN [13, 84, 85]. Although a role for AP1 in the transport of M6P receptors from TGN-to-endosome has not been ruled out, several studies have provided strong evidence that members of the clathrin-associated Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding (GGA) protein family mediate M6P receptor sorting into vesicles budding from the TGN [18, 86, 87]. The GGAs, which comprise three members in mammals (GGA1, GGA2 and GGA3) and two members in yeast (Gga1p and Gga2p), are monomeric, multi-domain, cytoplasmic adaptor proteins consisting of four domains: an amino-terminal VHS (for Vps27, Hrs, STAM homology) domain, a GAT (for GGA and TOM homology) domain, a connecting hinge segment, and a carboxyl-terminal GAE (for γ-adaptin ear homology domain) domain [18, 85, 86, 88, 89]. The GAT domain binds ADP-

ribosylation factor-GTP complexes and mediates recruitment of GGAs from the cytosol onto the TGN. The VHS domain interacts specifically with the AC-LL motif in the cytoplasmic tails of the M6P receptors. The GAE domain binds a subset of the accessory factors that interacts with the ear domain of AP-1, whereas the recruitment of clathrin triskeletons to budding vesicles is most likely mediated through clathrin-binding motifs of the hinge and GAE domain [18, 86, 87, 90]. Taken together, these findings suggest that GGAs are sorting proteins that recruit IGF-II/M6P receptors into clathrin-coated vesicles at the TGN for their transport to endosomes [18, 91]. On the other hand, receptor recycling back to the TGN from endosomes appears to involve an interaction between the cytoplasmic tail of the receptor and a complex of adaptor proteins termed as retromers. The retromers are composed of two subcomplexes: one consisting of Vps35p, Vps29p and Vps26p protein involve in cargo selection and the other comprising SNX1/SNX2/-SNX5-SNX6 dimers that regulate the formation of membrane tubules [92]. Several other proteins have also been implicated in the retrieval of the IGF-II/M6P receptor, including AP1, tail-interacting protein of 47 kDa (TIP47) and phosphofurin acidic cluster-sorting protein 1 (PACS-1) [18, 85, 93]. There is evidence that PACS-1 acts as a connector between the IGF-II/M6P receptor and AP1 to facilitate recycling of the receptor from early endosomes to the TGN [26], whereas TIP47 recycles receptors from late endosomes by binding Rab9, a late endosome GTPase [13, 18].

### 3.2. IGF-II/M6P receptor in the clearance/activation of extracellular ligands

Earlier studies using a variety of cultured cells, including rat adipocytes [94], L6 rat myoblasts [95], rat C6 glial cells [46] and mouse L cells [96] have demonstrated that IGF-II/M6P receptor mediates internalization and subsequent degradation of IGF-II. This is further substantiated by genetic experiments [97–99], which showed that deletion of the IGF-II/M6P receptor gene in mice led to prenatal death, but the fetuses were ~30% larger than normal [99] with increased levels of serum IGF-II but no alteration in IGF-II mRNA expression [97]. Interestingly, this phenotype can be rescued by simultaneous disruption of the genes for either IGF-II itself or the IGF-I receptor [98], thus indicating that increased levels of local IGF-II in the absence of IGF-II/M6P receptors produce a proliferative and hypertrophic response mediated *via* the IGF-I receptor.

In addition to IGF-II, IGF-II/M6P receptors regulate the internalization followed by degradation or activation of a variety of other ligands, including proliferin (a prolactin-related murine protein) [39], glycosylated human LIF [38], pre-prorenin [100] and epidermal growth factor receptor [101]. The cell-surface IGF-II/M6P receptor is believed to facilitate activation of latent TGF-β [102, 103], the precursor of the potent growth effector that regulates differentiation and growth of many cell types, by complex interactions involving IGF-II/M6P receptor, plasminogen and urokinase-type plasminogen activator receptor (uPAR). The ability of plasminogen and uPAR to bind the IGF-II/M6P receptor at distinct sites (localized to domain 1) from the latent TGF-β provides a plausible model in which urokinase binding to uPAR first facilitates the conversion of plasminogen to plasmin, which in turn proteolytically activates IGF-II/M6P receptor-bound TGF-β precursor [18, 42].

### 3.3. IGF-II/M6P receptor in mediating the biological effects of IGF-II

Unlike its function as a clearance receptor (in the clearance of IGF-II), the role of the IGF-II/M6P receptor in mediating certain biological effects of IGF-II by triggering an intracellular signaling cascade remains controversial. Because the IGF-II/M6P receptor lacks intrinsic catalytic activity, most of the biological effects of IGF-II have been attributed either to the IGF-I receptor [13] or IR-A [8]. Nonetheless, a number of studies over the years have suggested that certain biological effects of IGF-II are being triggered directly *via* the IGF-II/M6P receptor, including calcium influx in mouse embryo fibroblasts [104] and rat calvarial osteoblasts [105], increased protein phosphorylation [106] and alkalinization in proximal renal tubule cells [107], stimulation of $Na^+/H^+$ exchange and inositol trisphosphate production in canine kidney cells [108], increased amino acid uptake in muscle cells [109], increased glycogen synthesis in hepatoma cells [110], proteoglycan synthesis in human chondrosarcoma cells [111], calcium mobilization in rabbit articular chondrocytes [112], cell motility in rhabdomyosarcoma cells [113, 114], aromatase activity in placenta cytotrophoblasts [115], migration of human extravillous trophoblasts [116], insulin exocytosis by pancreatic β cells [117], and regulation of endogenous acetylcholine and GABA release from the adult rat brain [118, 119].

Since the cytoplasmic tail of IGF-II/M6P receptor lacks an intrinsic kinase domain, the intracellular mechanisms by which the receptor can mediate the range of biological effects listed above remain unclear. However, a number of studies using cell-free experimental systems or live cells have provided evidence for an interaction of the IGF-II/M6P receptor with heterotrimeric G proteins [72, 118, 120]. This is supported by the evidence that i) a cytoplasmic 14-residue region ($Arg^{2410}$-$Lys^{2423}$) of the human IGF-II/M6P receptor, which exhibits some sequence similarity with mastoparan - a small peptide in wasp venom that can directly activate Gi/Go proteins, is able to activate $G_{i\alpha}$ protein [121, 122], and ii) the existence of sequence homology between the C-terminal $Ser^{2424}$-$Ile^{2451}$ region of the IGF-II/M6P receptor and part of the pleckstrin homology domain of several proteins that bind $G_{\beta\gamma}$ and inhibit its stimulatory action on adenylyl cyclase activity [120]. At the functional level, there is evidence to suggest that IGF-II, acting *via* a G protein, can stimulate $Ca^{2+}$ influx in 3T3 fibroblasts and CHO cells [123, 124], increase exocytosis of insulin from pancreatic β cells [117], promote migration of extravillous trophoblast cells [116], and enhance PKC-induced phosphorylation of intracellular proteins [72]. Notwithstanding these results, the IGF-II/M6P receptor, under certain conditions, failed to interact with G protein or to couple with a $G_{i\alpha}$ in some studies [125, 126], thus raising doubt about the physiological relevance of the interaction between the IGF-II/M6P receptor and G proteins.

We have earlier shown that activation of the IGF-II/M6P receptor by $Leu^{27}$IGF-II, an IGF-II analog that preferentially binds to the IGF-II/M6P receptor as opposed to the IGF-I or insulin receptors, can induce depolarization of the basal forebrain cholinergic neurons and potentiate acetylcholine release from the adult rat hippocampus by a G protein-sensitive, PKCα-dependent pathway [118, 127]. More recently we reported that brain IGF-II/M6P receptors, as observed for several G-protein coupled receptors, are associated with β-arrestin 2. Following stimulation with $Leu^{27}$IGF-II, the receptors are translocated from detergent-resistant to detergent-soluble membrane fractions along with a portion of β-arrestin 2 [128].

Activation of the IGF-II/M6P receptor by IGF-II was also found to cause cardiac cell pathological hypertrophy *via* $G_{\alpha q}$-mediated increased phosphorylation of PKCα and calcium/calmodulin-dependent protein kinase II (CaMKII) [129]. Furthermore, IGF-II in cultured HEK293 cells can promote rapid membrane recruitment and activation of sphingosine kinase, leading to the production of extracellular sphingosine 1-phosphate (S1P), the ligand for G protein-coupled S1P receptors [130, 131]. This triple-membrane-spanning model of sphingosine kinase-dependent SIP receptor transactivation provides a general mechanism for the activation of G protein-dependent signaling pathways by non-classical G protein-coupled receptors [59, 131] but its physiological significance in relation to IGF-II/M6P receptor remains to be established.

### 3.4. IGF-II/M6P receptor in regulation of cell proliferation/death

It has long been suggested that overexpression of the IGF-II/M6P receptor acts as a growth inhibitor [132], whereas loss of the receptor function is associated with cell proliferation and progression of tumorigenesis [133–135]. This is supported by the evidence that IGF-II/M6P receptor plays a critical role in regulating the pericellular levels of IGF-II, a mitogen that is overexpressed in a number of human cancers [135, 136]. As stated above, the IGF-II/M6P receptor facilitates activation of the growth inhibitor TGF-β [42, 102, 137], and modulates the uptake/targeting of lysosomal enzymes, and glycosylated LIF [38], all of which require tight control to avoid the development and growth of tumors. Several studies have demonstrated that cytotoxic T cells kill target cells by granzyme B, which is taken up by perforin- and IGF-II/M6P receptor-based internalization mechanisms [138, 139]. Nevertheless, this conclusion has also been called into question [140]. Heterozygosity at the IGF-II/M6P receptor locus due to the loss of one allele and somatic mutations in the remaining allele have also been associated with a variety of cancers [141, 142]. Additionally, microsatellite instability within the IGF-II/M6P receptor gene has been shown to occur in a large number of gastrointestinal cancers [143], and single-nucleotide polymorphisms within the receptor gene have been suggested to increase the risk of developing cancers [144].

## 4. IGF-II/M6P receptor in neurodegenerative diseases

### 4.1. Distribution of IGF-II/M6P receptor in CNS

IGF-II/M6P receptor is widely but selectively distributed throughout the CNS (Table 2). Earlier studies using *in vitro* receptor autoradiography and membrane binding assays have shown the presence of specific $^{125}$I-IGF-II binding sites in various neuroanatomic regions of the brain, with particular enrichment in the choroid plexus, as well as in cortical areas, hippocampus, hypothalamus, cerebellum and certain brainstem nuclei of the adult rat brain [145–147]. A high to moderate density of specific $^{125}$I-IGF-II labeling, as revealed by *in vitro* receptor autoradiography, was apparent in various regions of the spinal cord [148]. Subsequent studies using Western blotting and immunohistochemistry have demonstrated that very high levels of the IGF-II/M6P receptor are expressed in the striatum, deeper layers (layers IV and V) of the cortex, pyramidal and granule cell layers of the hippocampus, selected thalamic nuclei, Purkinje cells of the cerebellum, pontine nucleus, and motor neurons of the brainstem as well as spinal cord [149–152]. Moderate neuronal labeling is noted primarily in the olfactory bulb, basal forebrain areas, hypothalamus, superior

colliculus, midbrain areas and granule cells of the cerebellum, whereas relatively low intensity of labeling is apparent in the outer layer of the cortex, *stratum lacunosum moleculare* of Ammon's horn, the molecular layer of the dentate gyrus and cerebellum [149–151]. Although most of the staining appears to be associated with neurons and their processes, non-neuronal ependymal cells also seem to express moderate levels of the receptor [149, 150]. Occasionally, IGF-II/M6P receptor immunoreactivity is evident in normal astrocytes, but its presence in resident microglia remains to be established [151, 152]. The distributional profile of the IGF-II/M6P receptor in the brain exhibits striking similarity with the distribution of the 46-kDa cation-dependent M6P receptor, but the relative intensity of the IGF-II/M6P receptor immunoreactivity was found to be greater, particularly in the basal forebrain and cerebral cortex, than that of the cation-dependent M6P receptor [153]. In keeping with protein profiles, high levels of IGF-II/M6P receptor mRNA have been demonstrated in various regions of the adult rat brain by Northern blot and RNAse protection assays [35, 154]. It is also of interest that IGF-II/M6P receptor expression in the brain is developmentally regulated, with high prenatal levels preceding a sharp postnatal decline, which is less acute in humans as compared to rat [155, 156]. The widespread distribution of the IGF-II/M6P receptor in the CNS suggests that one of its functions could relate to a "housekeeping" role in transporting intracellular or recapturing secreted lysosomal enzymes. The receptor may also participate in regulating the levels or functions of LIF, TGF-β and retinoic acid, which are known to modulate the activities of the nervous system [26, 102, 157, 158]. Additionally, the receptor may have a role in regulating the release of neurotransmitter/modulators [118, 119]. Several lines of experimental evidence suggest that IGF-II, which exhibits coordinated expression with the IGF-II/M6P receptor during development, has been shown to promote the growth, proliferation, and/or differentiation of a variety of neuronal phenotypes as well as glial cells under *in vitro* conditions [159–162]. Given the evidence that most, but not all, biological effects of IGF-II are mediated *via* IGF-I or insulin receptor, it is likely that mitogenic/growth-promoting effects of IGF-II during development are regulated by IGF-I receptor or insulin receptor, whereas the IGF-II/M6P receptor may act to stabilize or regulate local levels of IGF-II [26]. IGF-II/M6P receptor levels are also differentially altered following various surgical or pharmacological manipulations, thus indicating a potential role for the receptor in lesion-induced degenerative or regenerative processes [163–166]. As this review focuses primarily on recent developments on the potential role of the receptor in neurodegenerative diseases, especially in relation to Alzheimer's disease (AD), the reader is referred to several earlier reviews that describe the role of the receptor in regulating the functions of the brain [26, 127, 167, 168].

Unlike the normal brain, neurodegenerative diseases associated with aging constitute a set of pathological conditions characterized by progressive loss of neurons and synapses in selected areas of the nervous system. Brain cell death in degenerative disorders varies in type, location and rate of loss depending upon the disorders [169]. Even though it is unclear why certain brain regions are vulnerable in different degenerative disorders, it is also a fact that some neurons in targeted regions survive despite the loss of adjacent neurons [170]. The major neurodegenerative diseases that are associated with aging are AD, Parkinson disease (PD) and Huntington disease (HD). Other age-associated neurodegenerative diseases include

amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration, lysosomal storage diseases (LSDs), etc. The etiology of these diseases is either genetic and/or sporadic and the factors that trigger degeneration of neurons are considered to be multifactorial i.e., genetic, environmental, or endogenous factors related to aging. Nevertheless, neuronal loss in some of these diseases occurs *via* a shared mechanism triggered by an accumulation of misfolded toxic protein(s) - a possible consequence of abnormalities in synthesis, intracellular trafficking and/or clearance mechanisms [64, 171, 172]. This is partly supported by altered activity/functioning of the endosomal/lysosomal (EL) system which plays a critical role in the generation, and metabolism of proteins involved in various neurodegenerative diseases, including AD, PD and HD. Since IGF-II/M6P receptor is involved in the trafficking and sorting of lysosomal enzymes and some of their substrates to the EL system - the potential implication of this receptor in regulating the function of the EL system has been studied to a greater extent in relation to AD pathology, which shares several themes (such as protein misfolding) with other neurodegenerative diseases.

### 4.2. IGF-II/M6P receptor and AD pathology

AD is a progressive neurodegenerative disorder characterized by severe memory loss followed by deterioration of higher cognitive functions such as language, praxis, and judgment. Although in most cases AD develops sporadically after 65 years of age, a small proportion of cases corresponds to the early-onset (<65 years), autosomal-dominant form of the disease. Mutations in three genes, the amyloid precursor protein (*APP*) gene on chromosome 21, the presenilin 1 (*PSEN1*) gene on chromosome 14 and the presenilin 2 (*PSEN2*) gene on chromosome 1, have been identified as the cause of a large proportion of early-onset familial AD cases [173, 174]. Additionally, inheritance of the ε4 allele of the apolipoprotein E (*APOE*) gene on chromosome 19 increases the risk of late-onset/sporadic AD [175]. The neuropathological features associated with AD include the presence of extracellular β-amyloid (Aβ) peptide-containing neuritic plaques, intracellular tau-positive neurofibrillary tangles, and the loss of synapses and neurons in defined brain regions [176–178]. Structurally, neuritic plaques contain a compact deposit of proteinaceous amyloid filaments surrounded by dystrophic neurites, activated microglia, and fibrillary astrocytes. The principal component of neuritic/amyloid fibrils is the β-amyloid (Aβ) peptide, which is generated from APP [176, 177]. Pathological changes that characterize AD, together with the constitutive production of Aβ in the normal brain [179–181], indicate that an overproduction and/or a lack of degradation may increase Aβ levels which, in turn, contribute to neuronal loss and development of AD. Indeed, enhanced production of Aβ peptide has been associated with familial AD cases, whereas decreased clearance of the peptide has been linked to sporadic AD [182, 183].

The brain regions that are affected in AD include the basal forebrain, hippocampus, entorhinal cortex, neocortex and certain brainstem nuclei. Of all these regions, the basal forebrain, which provides the major cholinergic input to the hippocampus and the neocortex, is known to be most severely affected in AD pathology [184, 185]. Additionally, the loss of these neurons has been suggested to contribute to the progressive memory impairment associated with AD [185–187]. Given the evidence that the IGF-II/M6P receptor can potentiate acetylcholine release [118], enhance short- and long-term memory [188, 189], and

also regulate the function of the EL system, which is involved in Aβ metabolism [190, 191], we and others have evaluated levels/distribution of IGF-II/M6P receptor in brains of individuals with AD. In general, IGF-II/M6P receptor levels are not significantly altered in AD brains compared to age-matched controls but are found to be decreased in the hippocampus of patients with two copies of the *APOE* e4 alleles [192] or increased in the cortex of patients with *PSEN1* mutations [193]. Transgenic (Tg) mice expressing familial AD-linked mutant APP, on the other hand, showed increased levels/expression of IGF-II/M6P receptor in the affected regions of the brain at early but not late stages compared to age-matched controls [152] (unpublished data). Notwithstanding these results, IGF-II/M6P receptor is present in a subset of Aβ-containing neuritic plaques and activated astrocytes in both AD brains and mutant APP transgenic mice [152, 192], thus suggesting a potential role for the receptor in Aβ metabolism. In contrast to the receptor, IGF-II mRNA/peptide levels are decreased in AD brains [194, 195] and APP transgenic mice [195]. Additionally, enhancing IGF-II levels in the brain has been shown to ameliorate Aβ-containing neuritic plaques, synaptic deficits and cognitive impairments in two different lines of mutant APP transgenic mice [195, 196]. Since IGF-II can enhance working memory *via* the IGF-II/M6P receptor [188], it is likely that the receptor may have a role in regulating both cognitive functions and Aβ metabolism associated with AD pathology.

**4.2.1. IGF-II/M6P receptor and EL system in AD—**The EL system is part of the cell's central vacuolar system where secretory and membrane proteins/lipids are synthesized, modified, trafficked to appropriate cellular compartments and then eventually degraded. It is comprised of the endocytic pathway and lysosomal system including five major compartments: early endosomes, late endosomes, autophagic vacuoles, lysosomes and residual bodies [197]. Along the endocytic pathway, endosomes sort cargos through tubular protrusions for recycling or through intraluminal vesicles for degradation. Sorting of cargos involves fission of endosomal transport intermediate vesicles and fusion with other endosomes or with lysosomes [198]. Endosomes are able to regulate numerous pathways in the cell by sorting, processing, recycling, activating, silencing and degrading a variety of substances and receptors [199]. Lysosomes are intracellular organelles that contain about 60 enzymes crucial for degradation and recycling of macromolecules delivered by endocytosis, phagocytosis or autophagy [200]. Therefore, the EL system is not only important for protein trafficking and processing, but also the key component of the autophagy system involved in cellular homeostasis and protection [64, 201, 202].

A variety of experimental approaches over the last decade have indicated that the EL system, which acts as one of the hubs for APP metabolism, is drastically altered in "at risk" neurons of AD brains. The changes associated with early-endosomes, which precede the clinical symptoms and substantial deposition of Aβ peptides, include increased volumes and increased expression of proteins involved in endocytosis/ recycling (such as Rab5 and rababtin) as well as certain lysosomal enzymes. These alterations likely reflect increased rates of endocytosis and endosomal recycling of proteins involved in AD [190, 191]. Coincidentally, changes in the lysosomal system, which possibly occur after endosomal abnormalities, are reflected by a robust proliferation of lysosomes as well as expression of all classes of lysosomal hydrolases, including cathepsins B and D [203, 204]. Transgenic

mice overproducing Aβ peptides also exhibit an up-regulation of endosomal markers as well as lysosomal enzymes in selected brain regions [152]. Since cathepsins can directly influence APP processing and Aβ metabolism [205, 206], it is possible that enhanced levels of these enzymes may lead to intracellular accumulation of the peptides in vulnerable cells. This is partly supported by the evidence that pharmacological inhibition of cathepsin B or deletion of the cathepsin B gene has been shown to reduce Aβ burden in mutant APP transgenic mice [207, 208]. Additionally, as the leakage of lysosomal enzymes into the cytoplasm often leads to cell death [209, 210], it has also been suggested that chronic activation of lysosomes may have a role in the degeneration of neurons in AD brains. This notion is partly supported by two distinct lines of evidence i) increased levels of Aβ peptide trigger the release of lysosomal enzymes cathepsin D and β-hexosaminidase into the cytosol prior to degeneration of neurons [211, 212] and ii) preventing lysosomal breakdown protects neurons against toxicity [213]. At present, the mechanism underlying the activation of the EL system has not been clearly established, but it is likely to be regulated by receptors involved in the trafficking of molecules/enzymes to the lysosomes.

Previous studies have reported that IGF-II/M6P receptor levels, in general, are not markedly altered in most AD brain regions compared to controls [192, 193]. Conversely, the levels of the receptor are increased at early but not later stages of mutant APP-Tg mice [152] (unpublished data). Considering the multifunctional role of the IGF-II/M6P receptor, it is possible that unaltered receptor levels may reflect a rapid receptor turnover or a compromise in its other functions at the expense lysosomal enzyme delivery. Alternatively, a subset of the lysosomal enzymes could possibly be transported by other sorting receptors such as the cation-dependent M6P receptor or sortilin A receptor. In this regard, the cation-dependent M6P receptor, whose levels are elevated in vulnerable neurons of the AD brain, has been shown to redirect certain lysosomal hydrolases to early endosomes and increase the secretion of Aβ peptides in cultured fibroblasts [214, 215]. Thus further studies are required to define the relative significance of IGF-II/M6P receptor vs. cation-dependent M6P receptor in regulating the transport of lysosomal enzymes in AD pathology.

**4.2.2. IGF-II/M6P receptor in APP processing and Aβ metabolism—**Aβ peptides, the principal components of neuritic plaques, are a group of hydrophobic peptides containing 39–43 amino acid residues. These peptides are generated from proteolysis of APP, which is processed under normal conditions either by non-amyloidogenic α-secretase or amyloidogenic β-secretase pathways [190, 191]. The α-secretase pathway is mediated by a set of proteases that cleaves APP within the Aβ domain, yielding soluble APPα and a 10-kDa C-terminal fragment (CTF-α), which can be processed further by γ-secretase to generate $Aβ_{17-40}$/$Aβ_{17-42}$ [191]. The candidates that act as α-secretase are tumor necrosis factor-α converting enzyme (TACE or ADAM-17), ADAM-9, ADAM-10 or MDC-9. The amyloidogenic β-secretase pathway, on the other hand, cleaves APP to generate soluble APPβ and an Aβ-containing C-terminal fragment (CTF-β), which is further processed *via* γ-secretase to release full-length $Aβ_{1-40}$/$Aβ_{1-42}$ peptides [191]. While the β-secretase is a transmembrane aspartyl protease called BACE1, γ-secretase is a multi-transmembrane aspartyl protease complex made of presenilins (PS1 or PS2), nicastrin, PEN-2 and APH-1 [216–218]. Evidence suggests that non-amyloidogenic α-secretase processing occurs mostly

in the secretory pathway and at the cell surface, whereas amyloidogenic BACE1 processing of APP mainly occurs in the endocytic pathway [190, 191]. The relative efficiencies of the two alternative modes of APP processing in the brain can be influenced by a variety of factors including classical neurotransmitters, neuropeptides and growth factors.

Recently, using well-characterized mouse L-cells deficient in expression of the murine IGF-II/M6P receptor (MS cells) and corresponding MS9II cells that overexpress the human IGF-II/M6P receptor, we reported that overexpression of the IGF-II/M6P receptor increases the steady-state levels of *App*, *Bace1*, *Psen1, Ncstn* and *Aph1a* transcripts [219]. In addition, the levels of APP holo-protein and its cleaved products APP-CTFs (APP-CTFα and APP-CTFβ) were increased in MS9II cells, suggesting a potential role for the receptor in regulating the levels of APP and its processing. As for secretase, while BACE1 proteins were increased, the steady-state levels of PS1 and APH-1 were not altered in MS9II cells when compared to MS cells [219]. The activities of both β- and γ-secretases, as well as secretory $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ levels, were significantly higher in MS9II cells than MS cells [220]. Conversely, levels of the Aβ degrading enzyme IDE, but not neprilysin, were lower in MS9II cells indicating that overexpression of the IGF-II/M6P receptor may also influence clearance of Aβ-related peptides [220]. At the cellular level, subsets of APP, BACE1 and PS1 in the perinuclear region were found to be co-localized with IGF-II/M6P receptor in MS9II cells [220]. Additionally, receptor overexpression promoted APP localization in certain tubular structures, whose exact nature remains unclear. The significance of the IGF-II/M6P receptor overexpression on APP metabolism is highlighted by two lines of evidence i) siRNA-targeted decrease of the receptor level led to a concomitant reduction of APP, APP-CTFs and Aβ levels in MS9II cells and ii) activation of the receptor by its agonist $Leu^{27}$IGF-II did not significantly affect the levels or processing of the APP in MS9II cells [220]. Notwithstanding these results, the functional implications of normal levels of the IGF-II/M6P receptor on neuronal Aβ metabolism remain to be evaluated.

At present, the mechanism(s) by which IGF-II/M6P receptor overexpression regulates APP levels or processing remains unclear. A number of earlier studies have indicated that lipid-raft microdomains and non-raft regions of lipid bilayers play critical roles in the efficiency of amyloidogenic and non-amyloidogenic processing of APP, respectively [221–224]. Since the relative amount of full-length APP is found to be markedly higher in raft *vs* non-raft fractions in MS9II cells compared to MS cells, it is likely that IGF-II/M6P receptor overexpression may partly enhance Aβ production in MS9II cells by promoting raft microdomain association of APP [220]. Alternatively, given the evidence that IGF-II/M6P receptors are involved in the intracellular trafficking of lysosomal enzymes such as cathepsins B and D, which are known to regulate Aβ metabolism [59, 203, 225], it is possible that receptor overexpression can influence amyloidogenic processing of APP by altering the levels and/or redistribution of the enzymes within the EL compartments, as in the case of cation-dependent M6P receptor overexpression [214].

Evidence suggests that trafficking of both APP and BACE1 within the cells is intimately associated with Aβ production. Newly synthesized APP and BACE1 are first transported to the cell surface *via* the secretory pathway and then internalized into endosomes where BACE cleaves APP in an acidic environment leading to Aβ production. APP can also be

retrieved from endosomes back to TGN by binding to sortilin A receptor *via* PACS1, thus preventing its processing into Aβ peptides [191, 226]. Interestingly, retrograde transport of BACE1 from endosomes to TGN, as observed for IGF-II/M6P receptor, is mediated by binding to GGA proteins and the retromer complex [227, 228]. Dysfunction of the retromer complex not only causes endosomal accumulation of BACE1 but also increases production of APP-CTFβ and soluble APPβ [227, 229]. Since retrieval of the IGF-II/M6P receptor from endosomes to TGN overlaps with that of APP and BACE1, it is possible that overexpression of the receptor alters the trafficking dynamics in a manner that is conducive to amyloidogenic processing of APP within endosomal compartments leading to increased production of Aβ peptides.

**4.2.3. IGF-II/M6P receptor and the cholinergic system—**The IGF-II/M6P receptor is known to be widely but selectively distributed in various neuronal populations in the brain including the basal forebrain cholinergic neurons that are preferentially vulnerable in AD pathology [150]. Given the significance of the basal forebrain and acetylcholine in learning and memory processing, it has long been suggested that loss of these neurons and their innervations to the hippocampus and cortex contribute to the progressive memory impairment associated with AD patients [185]. Indeed, decreased levels of choline acetyltransferase activity, choline uptake and acetylcholine levels in the hippocampus and cortical regions of AD brains correlate positively with the clinical severity of dementia [185]. At present, however, the underlying cause of neurodegeneration of the basal forebrain cholinergic neurons remains unclear. Some earlier studies have shown that IGF-II/M6P receptor can enhance neuronal survival and increase the activity of acetylcholine transferase, the enzyme responsible for the synthesis of acetylcholine, in mouse primary septal cultured neurons [160]. N-nitrosodiethylamine-induced loss of neurons, on the other hand, has been shown to reduce both acetylcholine transferase and IGF-II/M6P receptor mRNAs [230]. We have reported that expression of the IGF-II/M6P receptor is increased in surviving cholinergic and non-cholinergic neurons in the basal forebrain region following *in vivo* administration of the immunotoxin 192 IgG-saporin [231]. Apart from influencing the viability of cholinergic neurons, there is evidence that activation of the IGF-II/M6P receptor can potentiate endogenous acetylcholine release from the hippocampus and cortex by a G protein-sensitive, PKCα-dependent pathway [118, 232]. Prenatal choline supplementation has also been shown to increase the expression of IGF-II and IGF-II/M6P receptor and enhance IGF-II-induced acetylcholine release in adult rat hippocampus and frontal cortex [233]. Thus, it would be of interest to determine whether the altered levels and/or signaling of the IGF-II/M6P receptor is the cause or consequence of the loss of basal forebrain cholinergic neuronal loss observed in AD brains.

**4.2.4. IGF-II/M6P receptor and memory enhancement in AD—**Apart from potentiating acetylcholine release, the IGF axis and the IGF-II/M6P receptor are involved in the retention and consolidation of memory processing [188, 189]. Using multiple approaches, it has recently been demonstrated that inhibitory avoidance learning in rat [188] and extinction learning in mouse [234] require increased synthesis of IGF-II. Administration of IGF-II directly into the hippocampus also enhanced memory retention and prevent forgetting [188]. This effect, which requires new protein synthesis, the function of the

activity-regulated cytoskeletal-associated protein, and glycogen-synthase kinase-3, is mediated *via* the IGF-II/M6P receptor [167, 188]. Intriguingly, systemic administration of IGF-II has also been shown to enhance the retention and persistence of working, short-term as well as long-term memories *via* activation of the IGF-II/M6P receptors [189]. In a parallel study, it has been shown that IGF-II acts as a downstream regulator of IκB kinase/nuclear factor of κB (IKK/NF-κB)-dependent synapse development and remodeling process. This effect, which appears to be mediated by the IGF-II/M6P receptor *via* the MEK/ERK signaling pathway, provides a morphological correlate of memory enhancement coupled to IGF-II [235]. The involvement of IGF-II in cognitive function is further reinforced by three lines of evidence: i) IGF-II mRNA/protein levels are decreased in the brains of AD patients as well as transgenic mice overexpressing mutant APP [194, 236], ii) direct administration or adenovirus-mediated delivery of IGF-II into the brain has been shown to ameliorate cognitive deficits as well as other AD-related pathology [195, 196], and iii) an IGF-II polymorphism has been associated with human cognitive function [237]. Collectively, these findings not only suggest a novel role for IGF-II in cognitive functions but also indicate the possibility that activation of the receptor by IGF-II or its mimetic may have therapeutic relevance in the treatment of AD pathology.

**4.2.5. IGF-II/M6P receptor and neuroprotection in AD—**Although IGF-II has been shown to protect neurons against a variety of toxic agents [238–241], the significance of the IGF-II/M6P receptor in mediating the effects of IGF-II remains unclear. A recent study, however, showed that ameliorating effects of IGF-II against glucocorticoid-induced toxicity can partly be mediated *via* IGF-II/M6P receptor [241]. A protective role for the receptor has also been suggested by the evidence that cultured PC12 cells that are resistant to Aβ-mediated toxicity [242] or brain neurons that survive 192 IgG-saporin-induced toxicity express high levels of the IGF-II/M6P receptors [231]. Additionally, TGFβ1, which is known to be activated by IGF-II/M6P receptor, has been shown to protect cultured neurons against Aβ-mediated toxicity both under *in vitro* and *in vivo* conditions [243–245]. Notwithstanding these results, overexpression of the IGF-II/M6P receptor was found to render MS9II cells more vulnerable to staurosporine-induced toxicity than IGF-II/M6P receptor lacking MS cells [220]. Thus, more work is needed to define the significance of the receptor in relation to degeneration of neurons observed in AD brains.

## 5. IGF-II/M6P receptor in lysosomal storage disorders (LSDs)

LSDs represent two groups of inherited metabolic neurodegenerative diseases triggered by a deficiency of lysosomal enzymes or components integral to lysosomal function, the mucolipidoses and the mucopolysaccharidoses [246]. Nearly two-thirds of the more than 50 LSDs involve the CNS, where neuronal dysfunction or loss results in mental retardation, progressive motor degeneration, and premature death [247, 248]. The majority of LSDs are caused by loss of function of lysosomal hydrolases [247], and most of these enzymes are transported by the IGF-II/M6P receptor. Dysfunction in any of these components may cause lysosomal deficits, leading to accumulation of degradative products and ultimately lead to LSD. I-cell disease (mucolipidosis type II) is one of the LSDs caused by deficiency of N-acetylglucosamine phosphotransferase, which catalyzes the first step in M6P addition to N-

linked oligosaccharides on the lysosomal enzymes, leading to defective lysosomal delivery of enzymes, secretion of those enzymes into the extracellular space, and accumulation of products destined to be degraded in dense inclusion bodies within cells [249]. Deficiencies of M6P-tagged lysosomal enzymes in some of the LSDs have been rectified by enzyme replacement therapy (ERT) where exogenously administered enzymes are delivered to the EL system *via* the cell-surface IGF-II/M6P receptor [250]. Recently discovered M6P analogs may improve both the affinity of the specific recombinant enzyme for the IGF-II/M6P receptor and the stability of the M6P moiety in the blood circulation and consequently enhance ERT efficacy and reduce enzyme dosage required for an efficient treatment [251, 252]. In addition, a newly-designed chimeric protein of IGF-II fused to β-glucuronidase to deliver enzyme *via* the IGF-II binding site on the IGF-II/M6P receptor can further increase the efficiency and improve delivery to resistant sites [253].

ERT has become well established as a useful therapeutic approach for several of the mucopolysaccharidoses (MPSs), a group of LSDs characterized by inherited deficiencies in a single lysosomal enzyme [250]. The effectiveness of this therapy depends on the expression of functional IGF-II/M6P receptors on the surface of the affected cells [250, 253]. The first disorder to be treated by this means was Gaucher disease, an inherited deficiency of the lysosomal enzyme glucocerebrosidase. Although there are now ERT approaches available for a number of the MPSs, including Fabry disease, Pompe disease, and MPS I, II and IV, the clinical effectiveness of these therapies has not matched the success attained with Gaucher disease. Even more problematic has been the nearly complete failure of ERT strategies to help correct brain dysfunction caused by CNS storage conditions in these diseases because the M6P-tagged replacement enzyme delivered intravenously does not transit the blood-brain barrier (BBB) [250, 253, 254]. The principal reason for this appears to be that the IGF-II/M6P receptors are not expressed on the BBB beyond the early perinatal period. Some hope for the application of brain-targeted ERT for the LSDs has been provided recently through the use of a chimeric form of sulphamidase coupled to the BBB-binding domain of apolipoprotein B [255]. The chimeric enzyme restored sulphamidase activity in the brains of sulphamidase-deficient mice with MPS IIIA. This highly engineered protein was taken up by binding of the apolipoprotein B portion of the chimera to the low-density lipoprotein receptor on the BBB and moved across into brain cells via transcytosis. Thus, in this case, the solution did not involve the IGF-II/M6P receptors.

Niemann-Pick disease covers a heterogenous group of three distinct lysosomal lipid storage diseases with autosomal recessive inheritance, i.e., Niemann-Pick type A, Niemann-Pick type B and Niemann-Pick type C (NPC). Niemann-Pick disease types A and B, caused by mutations in the gene coding for the lysosomal enzyme acid sphingomyelinase, result in the progressive accumulation of sphingomyelin and other lipids in the lysosomes of various tissues [256, 257]. NPC disease, which accounts for the majority of the cases of Niemann-Pick disease, is caused by mutation of either *NPC1* gene located on chromosome 18 or *NPC2* gene located on chromosome 14. This disease is characterized by a defect in intracellular cholesterol trafficking, which leads to accumulation of unesterified cholesterol in lysosomes. The buildup of cholesterol triggers hepatomegaly with foamy macrophage infiltration and chronic neurologic deterioration, leading to seizures, supranuclear ophthalmoplegia and progressive loss of motor and intellectual function.

Neuropathologically, impaired lipid trafficking results in the degeneration of neurons, activation of glial cells and the presence of intracellular neurofibrillary tangles [256, 258, 259]. Interestingly, the brains of patients with NPC contain increased levels of Aβ-related peptides and often display extracellular deposition of the peptide – thus exhibiting some striking similarity with AD brain pathology [260, 261].

Earlier studies have shown that altered levels of cholesterol can influence distribution/trafficking of IGF-II/M6P receptors within cells [262–264]. In fact, it has been reported that cholesterol accumulation in cultured cells induced by treatment with U18666A (an amphiphilic drug that induces an NPC-like phenotype at the cellular level) or siRNA-mediated NPC1 depletion can lead to redistribution of the IGF-II/M6P receptors to endosomes and impair its retrograde transport from late endosomes to the trans-Golgi network [262, 265, 266]. Studies from *Npc1*$^{-/-}$ mice, which recapitulate most of the pathological features of NPC disease, revealed that IGF-II/M6P receptor levels are not altered in the brain even though cathepsins B and D levels/activity are dramatically increased in the same regions [258, 267, 268]. At the cellular level, IGF-II/M6P receptor immunoreactivity is found to be somewhat decreased in neurons of the *Npc1*$^{-/-}$ mouse brains. Additionally, the majority of activated astrocytes, but not microglia, express higher levels of IGF-II/M6P receptor indicating that decreased neuronal levels may partially be compensated by glial expression of the receptor [268]. Since the subcellular distribution of the IGF-II/M6P receptor in *Npc1*$^{-/-}$ mouse brains has not yet been established, it remains unclear whether the increased levels/activity of the lysosomal enzymes may be associated with the altered levels/distribution of the receptor [258, 268].

## 6. IGF-II/M6P receptor in other neurodegenerative diseases

Since IGF-II/M6P receptor is widely distributed throughout the brain, it is expected that altered levels and/or functioning of the receptor, may have a role in various neurodegenerative disorders in addition to AD. PD, the second most common neurodegenerative disorder, is characterized by akinesia, rigidity, tremor and postural abnormalities. The sensorimotor symptoms are often accompanied by autonomic dysfunction, dementia, and cognitive deficits. Etiologically, PD is heterogeneous; only a minority (8–10%) segregates with genetic abnormalities, whereas the majority of cases are believed to be sporadic. The neuropathological features associated with PD include: i) loss of dopaminergic neurons of the substantia nigra pars compacta projecting to the striatum; and ii) the presence of intracellular inclusions known as Lewy bodies, composed of insoluble aggregates of a protein called α-synuclein, which plays a critical role in the degeneration of neurons [269]. It is reported that lysosomes and lysosomal enzyme cathepsin D are fundamental regulators of α-synuclein degradation through the chaperone-mediated autophagy pathway [270, 271]. Some recent studies have shown that several point mutations of Vps35, a subunit of retromer complex that plays an important role in the trafficking of the IGF-II/M6P receptor, can lead to the manifestation of late-onset PD in many ethnic groups [272–274]. Using a transgenic *Drosophila* model, it has been demonstrated that interference with the retromer function can lead to aberrant maturation of cathepsin D and subsequent accumulation of α-synuclein in the late-endosome/lysosome compartments [275]. Additionally, the missense Vps35 mutation (i.e., D620N) did not alter

steady-state levels of IGF-II/M6P receptor in the fibroblasts of PD patients, but triggered a deficit in retromer-dependent trafficking of the receptor and its ligand cathepsin D, which may underlie impaired degradation of α-synuclein resulting in the loss of neurons and development of PD pathology [276]. Interestingly, IGF-II/M6P receptor mRNA levels were not altered either in the frontal cortex or basal ganglia but decreased in the amygdala region of PD brains compared to control brains [277]. Although IGF-II/M6P receptor protein expression has not yet been evaluated in patients with PD, it appears that altered trafficking/function rather than levels of the receptor may have a role in the development of disease pathology. Impaired trafficking of the IGF-II/M6P receptor, as observed in PD-linked Vps35 mutation, may also contribute to the development of juvenile Batten disease - an autosomal recessive disorder with onset at 5–8 years of age. Hallmarks of this disease include lysosomal accumulation of undigested materials, degeneration of neurons in the brain and eye, blindness, seizures and development of cognitive and motor deficits, leading to death. This disease is caused by mutation of the gene encoding CLN3, a multipass transmembrane glycoprotein with unknown function. Using genetic manipulation, it has been shown that loss of CLN3 function triggers accumulation of IGF-II/M6P receptor in the trans-Golgi network, which leads to defective maturation/transport and activity of lysosomal cathepsins. Thus, dysfunction of lysosomal clearance mechanisms may underlie the development of devastating Batten disease [278].

Earlier studies indicated that $^{125}$I-IGF-II receptor binding was markedly increased in the spinal cords of patients with ALS - a fatal neurodegenerative disease caused by selective motor neuron death [279]. Using transgenic rat exhibiting loss of motor neurons, it has been demonstrated that IGF-II/M6P receptor expression is upregulated in reactive astrocytes towards the end-stages of disease pathology but not in pre-symptomatic animals suggesting a potential protective response against the loss of IGF-related trophic support [280]. In contrast to ALS, $^{125}$I-IGF-II receptor binding sites were not found to be altered either in the hippocampus or cerebellum following voluntary consumption of alcohol [146], but the receptor mRNA and binding sites are decreased in the cerebellar vermis and/or anterior cingulate gyrus of human alcoholics – often associated with loss of neurons as well as cognitive and motor deficits [281]. Multiple sclerosis (MS), an autoimmune inflammatory disease of the CNS, is characterized by demyelination, axonal loss, and neuronal death in selected brain regions. It is widely accepted that infiltration of inflammatory cells plays major roles in MS pathogenesis possibly *via* serine protease granzyme B secreted from granules of cytotoxic T cells [282, 283]. Interestingly, M6P treatment was found to attenuate granzyme B-mediated cell death by blocking the IGF-II/M6P receptor-dependent endocytosis, which suggests potential new targets for the treatment of MS [284]. However, no alteration was evident in $^{125}$I-IGF-II receptor binding sites in the cerebral cortex of MS patients [147], thus the significance of the receptor in the development of disease pathology, if any, remains to be determined. More recently, IGF-II/M6P receptor has been shown to be expressed highly in microglial nodules in human brains with human immunodeficiency virus encephalitis. Under an *in vitro* paradigm in which IGF-II/M6P receptor expression can be enhanced in microglia by interferon-γ, it has been shown to be a positive modulator of human immunodeficiency virus infection thus suggesting a contribution of the receptor in the persistence and spreading of viral-related pathology in the brain [285].

## 7. Conclusion

Recent studies have advanced our understanding of the structure, ligand binding properties and trafficking of the IGF-II/M6P receptor. This receptor plays a critical role in many cellular processes including growth and development, clearance of IGF-II, proteolytic activation of enzymes and growth factor precursors, apart from regulating trafficking of the lysosomal enzymes. This receptor, which is widely distributed throughout the brain, is also involved in IGF-II-mediated memory enhancement/ consolidation process as well as regulation of neurotransmitter release *via* G protein-dependent signaling mechanisms. Overexpression of the receptor has been shown to influence not only the functioning of the EL system but also the levels and metabolism of APP/Aβ-related peptides – thus suggesting its possible implication in AD pathology. Considering the significance of the receptor in regulating the trafficking of the lysosomal enzymes and other molecules, it is likely that the receptor may have a role not only in LSD but also in other neurodegenerative disorders that are associated with abnormal functioning of the EL system. However, further studies are needed to validate and fully characterize the significance of the IGF-II/M6P receptor in the normal brain and various neurodegenerative diseases to understand its full implications in regulating neuronal functioning.

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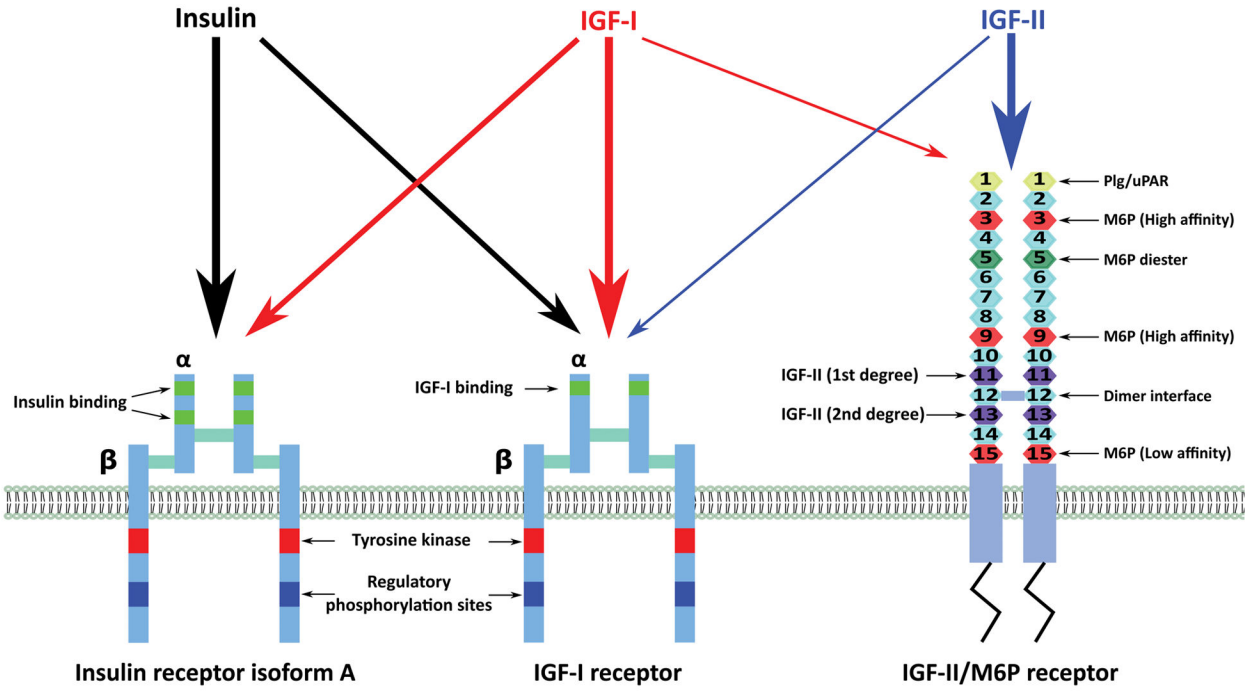


**Fig. 1. Structures of insulin receptor isoform A and the IGF-I and IGF-II/M6P receptors**

The IGF-I receptor and insulin receptor isoform A are members of the tyrosine kinase receptor family, which share high structural homology. Both receptors exist at the cell surface as a heterotetramer composed of two α and two β subunits joined by disulfide bonds. By contrast, the IGF-II/M6P receptor is a type I transmembrane glycoprotein dimer consisting of four structural domains, including an amino-terminal signal sequence, a large extracytoplasmic domain, a single transmembrane region and a carboxy-terminal cytoplasmic tail. Most ligands bind to the extracytoplasmic domain, and the IGF-II/M6P receptor could exist as dimers. The binding affinity of insulin, IGF-I and IGF-II to each of the three receptors differ from each other as indicated. Plg, plasminogen; uPAR, urokinase-type plasminogen activator receptor.

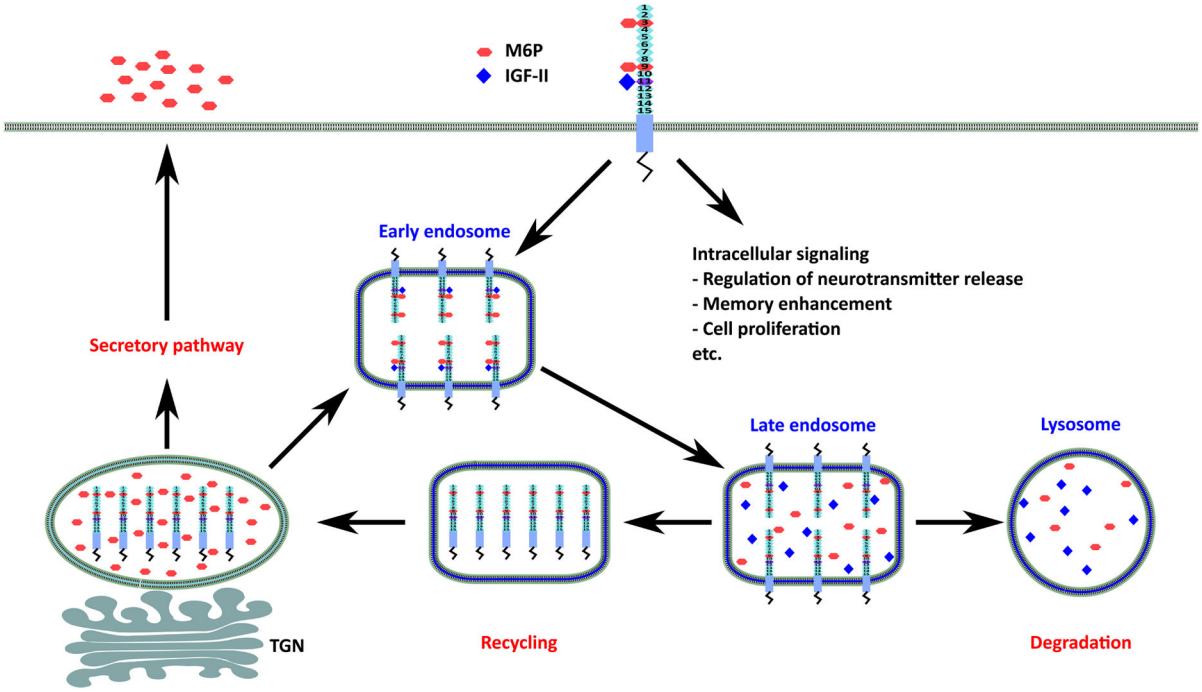


**Fig. 2. Cellular roles of IGF-II/M6P receptor**

Newly synthesized lysosomal enzymes are targeted within the trans-Golgi network for sorting to lysosomes by the posttranslational addition of M6P residues. IGF-II/M6P receptors, possibly interacting with GGA/AP-1, mediate the recruitment of lysosomal hydrolases to clathrin-coated vesicles, following which enzyme-receptor complexes are delivered to endosomal compartments. Lysosomal enzymes dissociate from M6P receptors within the low-pH environment of late endosomes and are subsequently delivered to lysosomes. Recycling of IGF-II/M6P receptors from late endosomes to the Golgi from early endosomes is thought to be mediated by retromer. Cell-surface IGF-II/M6P receptors also function in the capture and activation/degradation of extracellular M6P-bearing ligands, as well as in the clearance and degradation of the non-glycosylated IGF-II polypeptide hormone through clathrin-dependent endocytosis. IGF-II/M6P receptor may mediate intracellular signal transduction following IGF-II binding to regulate neurotransmitter release memory enhancement and cell proliferation.

## Table 1

Ligands that bind the IGF-II/M6P receptor and functional consequences of their binding

| Non-M6P-containing ligands | Consequences of IGF-II/M6P receptor binding |
|---|---|
| Insulin-like growth factor-II | Endocytosis and lysosomal degradation, possible signal transduction |
| Retinoic acid | Growth inhibition and/or apoptosis |
| Urokinase-type plasminogen | Participation in TGF-h activation at the cell surface; endocytosis and activator receptor lysosomal degradation |
| Plasminogen | Conversion to plasmin and participation of TGF-β activation |
| **M6P-containing ligands** | **Consequences of IGF-II/M6P receptor binding** |
| Lysosomal enzymes | Endocytosis and/or trafficking to lysosomes |
| Transforming growth factor- β precursor | Cell-surface proteolytic activation |
| Leukemia inhibitory factor | Endocytosis and lysosomal degradation |
| Proliferin | Induction of endothelial cell migration and angiogenesis |
| Thyroglobulin | Endocytosis and lysosomal activation and/or degradation |
| Renin precursor | Endocytosis and proteolytic activation and/or degradation |
| Granzyme A | Targeting to lytic granules and possible role in apoptosis |
| Granzyme B | Internalization and induction of apoptosis |
| DNAse I | Possible targeting to lysosomes |
| CD26 | Internalization and T cell activation |
| Epidermal growth factor | Endocytosis and lysosomal degradation |
| Herpes simplex viral glycoprotein D | Facilitation of viral entry into cells and transmission between cells |
| Varicella-zoster viral glycoprotein I | Facilitation of viral entry into cells |

## Table 2

Summary of the distribution of IGF-II/M6PR immunoreactivity in the adult brain

| Brain region | IGF-II/M6P receptor | | |
|---|---|---|---|
| Lateral septal nucleus | | + | |
| Medial septal nucleus | | +++ | |
| Nucleus of diagonal band of Broca | | +++ | |
| Caudate-putamen | | − to + | |
| Globus pallidus | | + | |
| Cerebral cortex, layers | I | II–III | IV–VI |
| Neocortex | + | + | +++ |
| Piriform/entorhinal | − to + | +++ | − to + |
| Amygdala | | +++ | |
| Stratum pyramidale | | ++ | |
| Strata oriens/radiatum | | − to + | |
| Molecular layer | | + | |
| Granule cell layer | | + | |
| Dorsal nuclei | | + | |
| Posterior nuclei | | + | |
| Ventral nuclei | | ++ | |

Relative intensity levels of the immunoreactivity for IGF-II/M6P receptor were estimated by visual inspection of stained sections under a light microscope. High (+++), intermediate (++), low (+), and undetectable (−) levels of immunoreactive intensity were discerned (Adapted from Konishi et al., 2005).