Mannose 6-Phosphate Receptors and Lysosomal Enzyme Targeting

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The targeting of lysosomal enzymes from their site of synthesis in the rough endoplasmic reticulum (RER) to their final destination in lysosomes is a multistep process requiring a series of interactions between cellular components and protein and carbohydrate recognition signals present on the lysosomal enzymes (1-6). These proteins share a common pathway with secretory proteins and membrane proteins during the early stages of their biosynthesis. All three classes of proteins contain a hydrophobic signal sequence that allows for their synthesis on membrane-bound polysomes in the RER and translocation into the lumen of the organelle. During this process the lysosomal enzymes as well as many secretory and membrane proteins are co-translationally glycosylated at selected asparagine residues. Following cleavage of the signal sequence and initial processing of asparagine-linked oligosaccharides, the proteins move by vesicular transport from the RER to the Golgi apparatus where they undergo a variety of post-translational modifications and are segregated from one another for targeting to their final destinations (7).

A key step in the sorting process is the generation of phosphomannosyl residues on the lysosomal enzymes. The phosphorylating enzyme recognizes a protein determinant shared by lysosomal enzymes, thereby selectively marking this class of proteins for subsequent segregation. The phosphomannosyl residues serve as high affinity ligands for binding to mannose 6-phosphate receptors (MPRs) in the Golgi. In this way the lysosomal enzymes are physically separated from proteins destined for secretion. The ligand-receptor complex then exits the Golgi via a coated vesicle and is delivered to a presynaptic acidic compartment where dissociation of the ligand occurs. The released lysosomal enzyme is packaged into a lysosome while the receptor either returns to the Golgi to repeat the process or moves to the plasma membrane where it functions to internalize exogenous lysosomal enzymes. Recent work has focused on the MPRs. Two different MPRs have been identified, characterized, and their cDNAs cloned. The routing of the receptors has been studied, and the determinants on the receptor that mediate trafficking are beginning to be defined. Some of the cellular components that interact with the receptors as they move from one compartment to the next have been identified. This review will summarize our current understanding of the MPRs and their biological functions.

Receptor Structure

The first MPR to be characterized was a membrane-associated glycoprotein with an apparent M, of 215,000. This receptor binds ligand independent of divalent cations. The other MPR is also a membrane-associated glycoprotein, but it has an apparent M, of 46,000 and requires divalent cations for optimal ligand binding. Both receptors show similar, but not identical, binding specificities toward phosphorylated oligosaccharides (8, 9). Based on their divalent cation requirements, we refer to the larger receptor as the cation-independent (CI) MPR and the smaller receptor as the cation-dependent (CD) MPR. The cloning of cDNAs for the CI-MPR from bovine (10), human (11, 12), and rat sources (13) has provided insights into the relationship between these two different proteins.

Sequence analyses, combined with proteolysis experiments of the receptor in membranes (16, 17), indicate that the bovine CI-MPR precursor consists of a 44-residue amino-terminal signal sequence, a 2269-residue extracytoplasmic domain, a single 23-residue transmembrane region, and a 163-residue carboxyl-terminal cytoplasmic domain (Fig. 1). The extracytoplasmic domain has 19 potential glycosylation sites, and at least two are utilized. Therefore, the size of the mature bovine receptor is likely to be between 275 and 300 KDa. The extracytoplasmic domain has a highly repetitive structure consisting of 15 contiguous units that have an average length of 147 amino acids. When the repeating units are compared to each other, numerous sequence identities are seen with the percent of identical residues ranging from 16 to 38%. In addition, there are numerous regions of conservatively substituted amino acids and a characteristic spacing of cysteine residues. The receptor is known to be phosphorylated (18, 19), and analysis of the cytoplasmic domain reveals sequences that are potential substrates for various protein kinases including protein kinase C and casein kinases I and II (13).

The predicted structure of the bovine CD-MPR consists of a 28-residue amino-terminal signal sequence, a 159-residue extracytoplasmic domain, a single 25-residue transmembrane region, and a 67-residue carboxyl-terminal cytoplasmic domain (Fig. 1). This receptor has five potential asparagine-linked glycosylation sites, four of which are utilized (14).

A comparison of the sequences of the two receptors reveals that they are related. The entire extracytoplasmic domain of the CD-MPR is similar to each of the repeating units of the CI-MPR, with sequence identity ranging from 14 to 28%. Thus, the CD-MPR is almost similar to the different repeating units of the CI-MPR as the repeating units are to each other. This similarity suggests that the two receptors share a common ancestry and that the CI-MPR arose from duplication of a single ancestral sequence. In contrast to these homologies, there are no significant primary sequence similarities between their signal sequences, transmembrane regions, or their cytoplasmic domains. However, the cytoplasmic domain of both receptors contains potential serine phosphorylation sites and clusters of acidic residues that are also found on other recycling receptors (20).

Chemical cross-linking experiments indicate that the CD-MPR is a dimer in the membrane (21, 22) and either a dimer or a tetramer in solution (23). The quaternary structure of the CI-MPR has not been analyzed in great detail, but hydrodynamic measurements are consistent with it being a monomer (24) while chemical cross-linking experiments in solution indicate that it may be an oligomer (21). Ligand binding studies have revealed that the CD-MPR binds 1 mol of the monovalent ligand Man-6-P and 0.5 mol of a divalent phosphorylated oligosaccharide/monomeric subunit (9). Therefore each functional dimer would have two Man-6-P binding sites, both of which can be occupied by a single oligosaccharide containing two Man-6-P residues. Evidence that each polypeptide monomer can fold into an independent ligand binding unit has been obtained by demonstrating that a truncated form of the bovine CD-MPR, which behaves as a soluble monomer in solution, is capable of binding Man-6-P (22). The CI-MPR, on the other hand, binds 2 mol of Man-6-P or 1 mol of a divalent phosphorylated oligosaccharide/monomer (8). This may indicate that only two of the 15 repeating segments of this receptor function in the binding of Man-6-P. While the identity of the binding segments has not yet been established, the two different proteolytic fragments of the CI-MPR encompassing repeating units 1-3 and 7-10 have been shown to bind a phosphorylated lysosomal enzyme. Thus the two functional Man-6-P binding domains are probably contained within these regions of the receptor.

Role of the Receptors in Sorting and Endocytosis

Two complementary experimental approaches indicate that the CI-MPR functions both in the sorting of newly synthesized lysosomal enzymes and in endocytosis of extracellular phosphorylated lysosomal enzymes. First, cultured cells that either lack endogenous CI-MPR (25) or are depleted of CI-MPR by treatment with anti-CI-MPR antisera (26, 27) secrete ~70% of their newly synthesized lysosomal enzymes and do not endocytose extracellular phosphorylated lysosomal enzymes. Second, the defective sorting and endocytosis pheno-
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The CI-MPR and the IGF-II Receptor Are the Same Protein

When Morgan et al. (11) cloned and sequenced the human insulin-like growth factor II (IGF-II) receptor, they made the surprising discovery that its sequence corresponds to that of the bovine CI-MPR. The identity of the CI-MPR and the IGF-II receptor has been confirmed by biochemical studies which show that this protein can bind phosphomannosyl residues and IGF-II, a nonglycosylated poly-enzymes. In these instances, IGF-II might act by inhibiting lysosomal enzyme binding to cell surface receptors thereby preventing the recapture of secreted lysosomal enzymes (34) or by altering the intracellular trafficking and distribution of the CI-MPR, possibly resulting in less efficient sorting in the Golgi (39). Interestingly, the chicken CI-MPR does not bind either human or chicken IGF-II (40). Nevertheless, chicken fibroblasts are highly responsive to IGF-II, possibly via binding to the IGF-1 receptor (41).

Other Growth Factors Bind to the MPR

Recently a number of secreted glycoproteins has been shown to contain the Man-6-P recognition marker (42-47). Presumably these ligands are secreted because they have a low affinity for the MPR and do not effectively compete with the bulk of lysosomal enzymes at the intracellular sorting site (38). These of these proteins have been identified as lysosomal enzymes that are secreted only under special circumstances (42-44). Another is porcine thyroglubulin which is secreted by thyroid follicle cells and then recaptured for degradation in lysosomes (45). The other proteins appear to be growth factors with no known lysosomal enzyme activity. Proliferin is a prolactin-related protein postulated to be an autocrine growth factor while transforming growth factor-11 precursor is the proform of a hormone that has multiple effects on cell growth and differentiation (46, 47). Both of these proteins can bind to the CI-MPR at the cell surface via their Man-6-P moieties. Their internalization could result in activation in endosomes or degradation in lysosomes. These findings indicate that the phosphomannosyl recognition system may have a broader biologic role than previously recognized.

Receptor Trafficking

Lysosomal enzymes can be targeted to the lysosome by either one of two pathways: a direct intracellular route ("biosynthetic pathway") or an endocytic pathway, with the former being the major pathway (Fig. 2). In the biosynthetic pathway, the formation of the active phosphomannosyl monoester on lysosomal enzymes occurs in the cis (early) Golgi compartment (1, 49). This raised the possibility that lysosomal enzymes might bind to the CI-MPR in the early Golgi and either pass through the Golgi as a complex or exit the Golgi at this point. Indeed, data consistent with the latter possibility have come from immunocytochemical studies demonstrating that in some, but not all, cells the CI-MPR is concentrated in the cis Golgi with very low levels in the trans (late) Golgi (50). However, sorting in most cells has been postulated to occur in a late Golgi compartment, based on the following observations. A number of lysosomal enzymes have been shown to contain terminally processed oligosaccharides (51, 52).

![Fig. 1. Schematic representation of the MPRs.](image)

![Fig. 2. Model for lysosomal enzyme targeting to lysosomes.](image)
indicating that these enzymes have traversed the entire Golgi complex since the glycosyltransferases responsible for terminal glycosylation reside in the trans Golgi elements (53). In addition, studies of the kinetics of receptor trafficking have demonstrated that the MPRs return to the last Golgi compartment, the trans Golgi network, much more frequently than they cycle to the early Golgi compartments (50, 54). Furthermore, CI-MPRs and lysosomal enzymes have been localized to clathrin-coated vesicles in the region of the trans Golgi network (55, 56). Taken together, these data indicate that lysosomal enzymes are sorted from other classes of proteins in the trans Golgi network (7).

Immunocalization studies and biochemical analyses reveal very low or undetectable amounts of the CI-MPR in lysosomes while, in contrast, a significant amount of the receptor is found in endosomal structures (18, 55, 57-59). This has led to the concept that Golgi-derived vesicles containing lysosomal enzyme-receptor complexes are delivered to acidic endosomal compartments rather than to lysosomes (57-60). The low pH of the endosomal compartment would cause the complex to dissociate and the released lysosomal enzymes could be packaged into lysosomes while the CI-MPRs could recycle out of this compartment. (The variation in the pH of the different compartments assures the proper vortual transport of ligands. The receptor binds ligand at the plasma membrane, but at acidic pH; the Golgi is near neutrality while the endosome is acidic. Consequently, the receptor will bind lysosomal enzymes in the Golgi and discharge them in endosomes.) Griffiths et al. (57) have described an acidic late endosomal structure in normal rat kidney cells which may serve as a site where lysosomal enzymes dissociate from the CI-MPR. Furthermore, the MPR is lost from a vesicular population of the trans Golgi network when the Golgi apparatus is damaged. These results indicate that the MPRs are involved in the recycling process. Griffiths and co-workers (56) have shown that receptor occupancy does not have a significant effect on the MPR trafficking. Alterations in the steady state distribution of the MPR relative to lysosomes have been shown to indicate that these enzymes have traversed the entire Golgi complex, contains lysosomal enzymes as well as a lysosomal membrane protein (lgl120), and is enriched in CI-MPRs. This structure is distinct from the trans Golgi network and early endosomes. Studies by Geuze et al. (58) also have provided evidence that the CI-MPR is segregated from lgl120 and presumably from lysosomal enzymes in early endosomal structures. However, it is currently unknown if the newly synthesized lysosomal enzymes are delivered to early endosomes, late endosomes, or both types of endosomes. The finding of CI-MPRs in early endosomes does not resolve this issue since these molecules may be derived from the plasma membrane.

Extracellular lysosomal enzymes may also be delivered to the lysosome via the endocytic pathway. A small proportion of lysosomal enzymes is typically secreted by cells (1, 2). Some of these enzymes may bind to cell surface CI-MPRs and be internalized via clathrin-coated pits and vesicles (55, 61). The internalized acid hydrolases enter acidified endosomal compartments where they dissociate from the CI-MPRs and are delivered to lysosomes. Measurements of the number and half-life of MPRs and the rate of ligand internalization indicate that the MPRs are reutilized and can undergo many rounds of ligand delivery (62). In addition, studies using antibodies (26, 60, 63) or galactosyltransferase (30) to label receptors on the cell surface indicate that the CI-MPRs in the trans Golgi region (65). The Golgi-derived adaptor proteins bind to the CI-MPR (100.47-19-kDa complex) has been isolated from clathrin-coated pits in the Golgi region (66). The Golgi-derived adaptor proteins bind to the CI-MPR and interact with the cytoplasmic tail of the CI-MPR, LDL receptor, and the poly-1g receptor, but not with a mutant CI-MPR cytoplasmic tail that lacks the two tyrosine residues at positions 24 and 26. These results indicate that tyrosine residues in the cytoplasmic tails of these endocytic receptors are necessary for their interaction with the plasma membrane-derived adaptor proteins and suggest that this interaction is what mediates the entry of selected receptors into clathrin-coated pits. A different set of adaptor poly peptides (100.47-19-kDa complex) has been isolated from clathrin-coated pits in the Golgi region (66). The Golgi-derived adaptor proteins bind to the CI-MPR but not to that of the LDL receptor that lacks the two tyrosine residues at positions 24 and 26. The mutant CI-MPR tail that lacked tyrosines still interacted with the Golgi-derived adaptor proteins. Thus, both classes of adaptor poly peptides are likely to be involved in the routing of the CI-MPR, with one set directing departure from the plasma membrane and the other set directing departure from the Golgi.

The identification of these two adaptor protein complexes, each of which is located at a unique site along the receptor's targeting pathway and interacts with distinct signals on the receptor, provides a first step toward understanding the mechanism by which the CI-MPR is routed. In addition, the selective recycling of the CI-MPR to the trans Golgi network has been reconstituted in vitro (70). The properties of receptor internalization in this cell system suggest that the CI-MPRs are routed via the clathrin-coated vesicular transport mechanism. The ability to reconstitute in vitro this portion of the CI-MPRs intracellular pathway should aid in the identification of the cellular components involved in this targeting process. Furthermore, the recent isolation of endosomal structures enriched in MPRs should be useful in the characterization of other molecules involved in routing the receptors (71).

**Potential Regulators of Receptor Trafficking**

Several studies have analyzed the effect of ligand binding on CI-MPR trafficking. Alterations in the steady state distribution of the CI-MPR have been found in some (72, 73) but not all (74, 75) studies where lysosomal enzyme synthesis is inhibited or where the dissociation of receptor-ligand complexes is prevented. On the other hand, studies of the kinetics of receptor movement under these conditions have shown that receptor occupancy does not have a significant effect
on the rate of receptor movement (30, 54, 76). These latter results indicate that the CI-MPR shuttles constitutively between the cell surface and intracellular compartments. However, the presence or absence of ligand might induce small changes in the rates of receptor movement to various compartments, thereby affecting the steady state distribution of receptor without having much of an effect on the overall rate of receptor movement. In some specialized cell types, insulin and other growth factors induce a dramatic redistribution of the CI-MPR and the glucose transporter from endosomal compartments to the cell surface (77, 78, and op. cit.). This redistribution is associated with a decrease in the phosphorylation state of the plasma membrane-associated CI-MPRs (78). It is not known if this change in phosphorylation is the cause or a consequence of the redistribution. This can now be tested directly by mutating the potential phosphorylation sites on the receptor and determining if the response to growth factors is maintained.

**Conclusions**

The cloning of the MPRs has provided new insight into the structure and function of the receptors, yet many questions remain. What is the purpose of having two distinct receptors for lysosomal enzyme trafficking? Are they redundant or do they function in the delivery of lysosomal enzymes to different compartments? Why does the same receptor bind lysosomal enzymes and IGF-II? Is lysosomal enzyme trafficking regulated by IGF-II? Does this receptor mediate signal transduction, or does it only function in the transport of lysosomal enzymes, IGF-II, and perhaps other unidentified ligands to the lysosome? What are the signals on the receptors that are involved in routing, and what are the cellular components that direct the receptor’s movement? Studies involving the expression of mutated MPRs, the interaction receptors with components of clathrin-coated pits, and the in vitro reconstitution of vesicular transport between the various compartments are all contributing to the understanding of this transport system at the molecular level.

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