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REVIEW

Strategies for carbohydrate recognition by the mannose 6-phosphate receptors

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The two members of the P-type lectin family, the 46 kDa cation-dependent mannose 6-phosphate receptor (CD-MPR) and the 300 kDa cation-independent mannose 6-phosphate receptor (CI-MPR), are ubiquitously expressed throughout the animal kingdom and are distinguished from all other lectins by their ability to recognize phosphorylated mannose residues. The best-characterized function of the MPRs is their ability to direct the delivery of ∼60 different newly synthesized soluble lysosomal enzymes bearing mannose 6-phosphate (Man-6-P) on their *N*-linked oligosaccharides to the lysosome. In addition to its intracellular role in lysosome biogenesis, the CI-MPR, but not the CD-MPR, participates in a number of other biological processes by interacting with various molecules at the cell surface. The list of extracellular ligands recognized by this multifunctional receptor has grown to include a diverse spectrum of Man-6-P-containing proteins as well as several non-Man-6-P-containing ligands. Recent structural studies have given us a clearer view of how these two receptors use related, but yet distinct, approaches in the recognition of phosphomannosyl residues.

Keywords: lectin/lysosome/mannose 6-phosphate receptor/protein targeting

Introduction

Lysosomes require a repertoire of over 60 different acid hydrolases to carry out the degradative metabolism of proteins and other macromolecules. Delivery of newly synthesized soluble acid hydrolases to lysosomes is carried out by the P-type lectins, the cation-dependent mannose 6-phosphate receptor (CD-MPR) and cation-independent mannose 6-phosphate receptor (CI-MPR), and is dependent upon specific recognition in the *trans*-Golgi network (TGN) of Man-6-P residues on their *N*-linked oligosaccharides and their subsequent removal from the secretory pathway. The importance of this targeting process in the generation of lysosomes containing a full complement of hydrolytic enzymes is evidenced by the existence of over 40 different lysosomal storage diseases (Neufeld 1991; Futerman and van Meer 2004; Vellodi 2004). Lysosomal storage diseases constitute a significant portion of inborn metabolic disorders although individually rare, the collective frequency of these disorders is estimated to be approximately 1 in 5000 live births (Meikle and Hopwood 2003). The majority of lysosomal storage diseases are caused by a deficiency of a single hydrolytic enzyme that results in the accumulation of undigested endogenous macromolecules within lysosomes.

The MPRs have a relatively long half-life ($t_{\frac{1}{2}} \sim 20$ h) as they cycle continuously between the TGN, endosomes, and the plasma membrane where they load and unload their cargo. Both receptors display optimal ligand binding at pH ∼ 6.5 and no detectable binding below pH ∼ 5, which is consistent with their function of binding lysosomal enzymes in the TGN and subsequently releasing their ligands in the acidic environment of endosomes. The lysosomal enzymes are packaged into lysosomes (Le Borgne and Hoflack 1998; Mullins and Bonifacino 2001), whereas the unoccupied MPRs either recycle back to the TGN to retrieve additional enzymes from the secretory pathway or move to the plasma membrane. In contrast to many endocytic receptors, only ∼10% of the MPRs are present at the cell surface while the remainder of the receptors are found predominantly in endosomal compartments and the TGN (for reviews, see Dell' Angelica and Payne 2001; Mullins and Bonifacino 2001; Ghosh et al. 2003). Cell surface CI-MPRs, but not CD-MPRs, carry out the internalization of a variety of Man-6-P-containing ligands for their subsequent clearance or activation (see below). With respect to lysosomal enzymes, cell surface CI-MPRs function in the endocytic recapture of Man-6-P-modified acid hydrolases that are not properly sorted from the secretory pathway at the TGN (Ludwig et al. 1994; Pohlmann et al. 1995; Kasper et al. 1996; Munier-Lehmann et al. 1996). This property of the CI-MPR forms the basis of enzyme replacement therapy for several lysosomal storage diseases (Brady 2006) and explains, in part, the observation that the CI-MPR is more efficient than the CD-MPR in targeting lysosomal enzymes to the lysosome (Watanabe et al. 1990; Munier-Lehmann et al. 1996). Although the CD-MPR cycles between the cell surface and intracellular compartments, it does not play a significant role in the recapture of secreted lysosomal enzymes due to its decreased ability to bind lysosomal enzymes at pH 7.4 (Tong and Kornfeld 1989).

Although the CD-MPR and CI-MPR are the only two lectins that have been shown to bind phosphorylated mannose residues with high affinity, a few proteins have been identified that contain a mannose 6-phosphate receptor homology (MRH) domain (Munro 2001). Several of these MRH-containing proteins have been implicated in carbohydrate recognition events. However, definitive studies are lacking with respect to their ability to interact directly with a specific glycan structure. This review will

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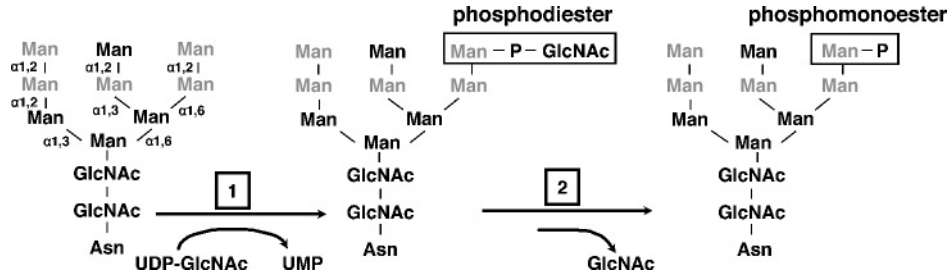

Fig. 1. Generation of the Man-6-P tag on *N*-linked oligosaccharides. Phosphorylation of mannose residues on *N*-linked oligosaccharides occurs in two steps. First, the GlcNAc-phosphotransferase transfers GlcNAc-1-phosphate from UDP-GlcNAc to the C-6 hydroxyl group of mannose to form the Man-P-GlcNAc phosphodiester intermediate. Second, the uncovering enzyme removes the GlcNAc moiety in the TGN, revealing the Man-6-P phosphomonoester. The five potential sites of phosphorylation are indicated (*gray*).

focus on the extracellular region of the MPRs and will highlight the findings obtained from recent structural studies that have revealed how these two receptors interact with phosphomannosyl residues.

Generation of Man-6-P on *N*-linked oligosaccharides

Synthesis of the Man-6-P signal occurs by a two-step process during transit of lysosomal enzymes through the endoplasmic reticulum (ER)-Golgi biosynthetic pathway. The first enzyme, UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (GlcNAc-phosphotransferase; IUBMB accession number EC 2.7.8.17), attaches GlcNAc-1-phosphate to the C-6 hydroxyl group of one or more mannose residues to form the Man-P-GlcNAc phosphodiester intermediate (Hasilik et al. 1980; Tabas and Kornfeld 1980; Varki and Kornfeld 1980; Waheed et al. 1982) (Figure 1). This selective phosphorylation of *N*-linked high mannose-type oligosaccharides in the *cis*-Golgi (Pohlmann et al. 1982; Lazzarino and Gabel 1988) is achieved by the ability of the GlcNAc-phosphotransferase to recognize a surface patch on lysosomal enzymes, with two or more lysine residues that are correctly spaced relative to each other and to the oligosaccharide chain serving as critical elements of a more extensive three-dimensional recognition marker (Reitman and Kornfeld 1981; Warner et al. 2002; Steet et al. 2005). The second enzyme, *N*-acetylglucosamine-1-phosphodiester α-*N*-acetylglucosaminidase, which is often referred to as the "uncovering enzyme" (IUBMB accession number EC 3.1.4.45) removes the GlcNAc moiety in the TGN to generate the phosphomonoester (Varki and Kornfeld 1981; Waheed et al. 1981; Varki et al. 1983) (Figure 1). An interesting aspect to the regulation of phosphomonoester synthesis is that the uncovering enzyme, which is localized in the TGN, is synthesized as a pro-enzyme, and the removal of the propeptide by furin in the TGN has been shown to be essential for the generation of an active enzyme (Rohrer and Kornfeld 2001; Do et al. 2002). The MPRs encounter a diverse array of Man-6-P-tagged lysosomal enzymes in the TGN since phosphorylated oligosaccharides isolated from lysosomal enzymes have been shown to be quite heterogeneous, containing one or two phosphomannosyl residues (phosphomonoester or GlcNAc phosphodiester) that can be located at five different positions in the oligosaccharide chain (Figure 1) (Hasilik et al. 1980; Tabas and Kornfeld 1980; Varki and Kornfeld 1980).

Genetic defects in the GlcNAc-phosphotransferase cause the lysosomal storage disease, mucolipidosis II (ML II; also referred to as "I-cell disease") and mucolipidosis III (ML III; also referred to as "pseudo-Hurler polydystrophy") in which the activity of the enzyme is absent or reduced, respectively (Kudo et al. 2006). Unlike most lysosomal storage diseases which affect single enzymes in a catabolic pathway, these disorders are characterized by impaired sorting of multiple newly synthesized enzymes to lysosomes and their subsequent secretion from cells due to the inability of the enzymes to acquire the Man-6-P tag and be recognized by the MPRs. ML II is more severe than ML III, with ML II patients typically dying during the first decade of life due to dysfunction of the cardiovascular system resulting from progressive storage of undigested material in the lysosomes of fibroblasts within the connective tissue of heart valves, endocardium, myocardium, and perivascular areas (Kornfeld and Sly 2001).

The discovery of the MPRs originated from investigations into the molecular basis of ML II. Hickman and Neufeld (1972) made key observations that ML II fibroblasts were capable of endocytosing lysosomal enzymes secreted by normal cells while, in contrast, normal cells were incapable of internalizing the enzymes secreted by ML II fibroblasts. Their suggestion that lysosomal enzymes contained a recognition marker required for uptake and transport to lysosomes was later confirmed with the identification of the marker as Man-6-P (Kaplan et al. 1977; Distler et al. 1979; Natowicz et al. 1979). Analyses of fibroblasts derived from transgenic animals lacking both MPRs show that these cells secrete the majority of their lysosomal enzymes and accumulate undigested material in their lysosomes in a manner similar to that observed in fibroblasts from ML II patients (Ludwig et al. 1996; Dittmer et al. 1998; Schellens et al. 2003), demonstrating that a similar phenotype can result from either a deficiency of the Man-6-P tag or the MPRs.

Expanding list of Man-6-P-containing proteins

In addition to lysosomal enzymes, the repertoire of identified extracellular ligands of the CI-MPR has expanded to include a diverse spectrum of Man-6-P-containing proteins (Dahms and Hancock 2002). For example, two viruses, herpes simplex virus (Brunetti et al. 1994) and varicella–zoster virus (Gabel et al. 1989), have been shown to express viral Man-6-P-containing glycoproteins which function to facilitate the entry of the virus into mammalian cells via the CI-MPRs. Elegant studies by Chen

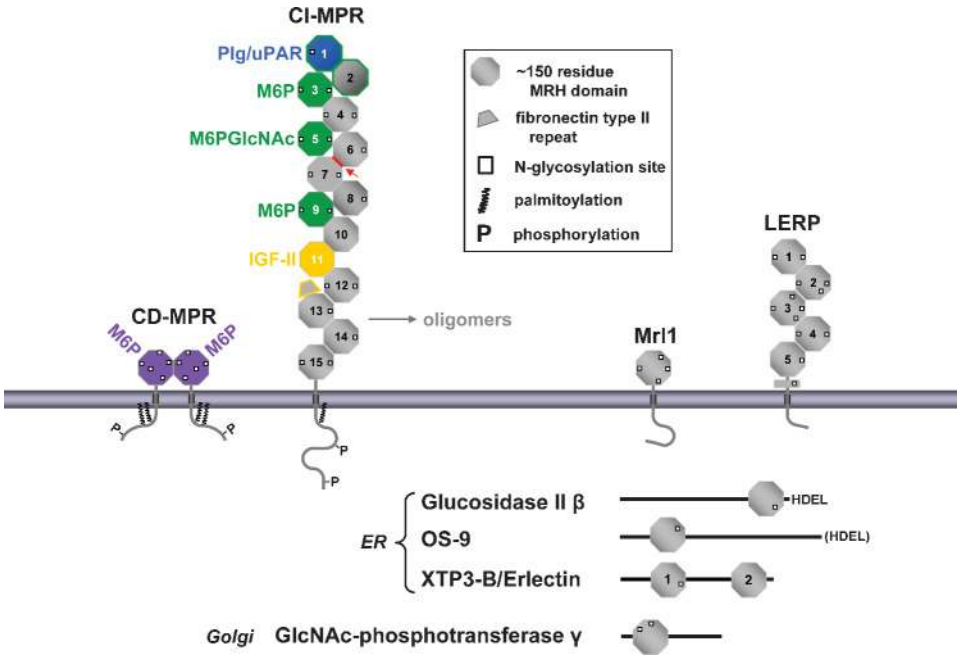

Fig. 2. Schematic diagram of the MRH domain containing proteins. The MPRs, 381-residue Mrl1, and 886-residue LERP are type I transmembrane glycoproteins. The 279-residue CD-MPR exists as a homodimer. The 2499-residue CI-MPR also undergoes oligomerization and most likely exists as a dimer. The Man-6-P binding sites of the CD-MPR (*purple*) and CI-MPR (*green*) are indicated. Domains 1 and 2 are outlined in *green* since the presence of these two domains enhances the affinity of domain 3 for lysosomal enzymes by ~1000-fold (Hancock, Yammani, et al. 2002). The CD-MPR contains a single high affinity Man-6-P binding site per polypeptide. In contrast, the CI-MPR contains three carbohydrate recognition sites: two high affinity sites are localized to domains 1–3 and domain 9 and one low affinity site is contained within domain 5. The IGF-II (*gold*) and plasminogen (Plg)/uPAR (*blue*) binding sites are also indicated. The fibronectin type II repeat present in domain 13 is outlined in *yellow* since its presence increases the affinity of domain 11 for IGF-II by ~10-fold. The *red arrow* indicates the location of a proteolytically sensitive cleavage site between domains 6 and 7 (Westlund et al. 1991). The 528-residue glucosidase II β subunit, 667-residue OS-9, and 483-residue XTP3-B/Erlectin are soluble resident ER proteins. Glucosidase II β subunit and the yeast ortholog of OS-9 contain a C-terminal ER retention signal (HDEL). The 305-residue GlcNAc-phosphotransferase γ subunit, in complex with the catalytic α/β subunits, is enriched in *cis*-Golgi cisternae.

et al. (2004) have clarified the pathology of varicella–zoster virus infection. Intracellular CI-MPR diverts newly enveloped varicella–zoster virus to late endosomes, thereby preventing the spread of the virus. However, CI-MPR expression is lost in maturing superficial epidermal cells and thus these cells do not divert the virus to endosomes but rather constitutively secrete infectious virus particles. With respect to endogenous mammalian proteins, a number of secreted proteins have been shown to contain Man-6-P, such as transforming growth factor-β (TGF-β) precursor (Purchio et al. 1988), the cytokine leukemia inhibitory factor (Blanchard et al. 1998), the placental angiogenic hormone proliferin (Lee and Nathans 1988), the aspartic protease renin precursor (Faust et al. 1987), the serine proteases granzymes A and B (Griffiths and Isaaz 1993), the T-cell activation antigen CD26 (Ikushima et al. 2000), cellular repressor of E1A-stimulated genes (Journet et al. 2000; Di Bacco and Gill 2003), and the cysteine proteinase inhibitor cystatin F (Journet et al. 2002). Roles for the interaction of these nonlysosomal proteins with cell surface CI-MPRs include activation (e.g., TGF-β precursor (Dennis and Rifkin 1991), renin precursor (van Kesteren et al. 1997)) and clearance from the plasma (e.g., leukemia inhibitory factor (Blanchard et al. 1999)). Recent proteomic approaches using affinity columns containing immobilized CI-MPR have identified new Man-6-P-containing lysosomal proteins as well as other proteins that acquire the Man-6-P modification (Sleat et al. 2005, 2006, 2007; Czupalla et al. 2006). For example, this powerful methodology has demonstrated that a small proportion of abundant classical plasma proteins (e.g., α1-acid glycoprotein, ceruloplasmin, haptoglobin) exist as Man-6-P-containing glycoforms (Sleat et al. 2006). Although not directly demonstrated, it is assumed that these nonlysosomal proteins acquire Man-6-P by the same GlcNAc-phosphotransferase that acts on acid hydrolases. It is also not clear how these Man-6-P-containing proteins can escape interaction with the MPRs in the TGN, so that they are secreted rather than being diverted to the endosome/lysosome system. Furthermore, as many of the studies have been performed on recombinantly expressed proteins, it is not known what percentage of the endogenous proteins carry the Man-6-P modification. Additional studies are needed to probe the biosynthesis and trafficking of these nonlysosomal MPR ligands.

Primary structure of the MPRs

The MPRs are type I transmembrane glycoproteins that exist as oligomers: the CD-MPR is a stable homodimer and the CI-MPR most likely exists in the form of a dimer (for review, see Dahms and Hancock (2002)) (Figure 2). The bovine CD-MPR is composed of a 28-residue amino-terminal signal sequence, a 159-residue extracytoplasmic region, a 25-residue transmembrane region, and a 67-residue carboxyl-terminal cytoplasmic domain. The extracytoplasmic region of the CD-MPR contains 6 cysteine residues that are involved in the formation of three intramolecular disulfide bonds that play an essential role in the folding of the receptor (Wendland, von Figura, et al. 1991). The bovine

CI-MPR contains a 44-residue amino-terminal signal sequence, a large 2269-residue extracytoplasmic region, a 23-residue transmembrane region, and a 163-residue carboxyl-terminal cytoplasmic domain. The large extracytoplasmic region is composed of 15 contiguous domains that display a similar size (~150 residues) and cysteine distribution, and exhibit significant amino acid identity (14–38%) when compared to each other and to the CD-MPR (Figure 3A), giving rise to the prediction that they have a similar tertiary structure. This hypothesis has been confirmed, in part, by crystal structure determinations which show that the extracytoplasmic region of the CD-MPR and domains 1, 2, 3, 11, 12, 13, and 14 of the CI-MPR all exhibit the same fold (Olson, Dahms, et al. 2004; Olson, Yammani, et al. 2004; Brown et al. 2008) (see below). Except for domain 13 of the CI-MPR which has 12 cysteine residues due to a 43-residue insertion homologous to the type II repeat of fibronectin, each of the domains of the CI-MPR that has been crystallized to date contains 8 cysteine residues that form four intramolecular disulfide bridges. The MPRs undergo several types of co- and posttranslational modifications, including *N*-glycosylation, palmitoylation, and phosphorylation (Figure 2). Palmitoylation of the CD-MPR has been shown to prevent its degradation in lysosomes (Rohrer et al. 1995) while serine phosphorylation of both MPRs has been shown to influence their intracellular transport (Braulke and Mieskes 1992; Meresse and Hoflack 1993; Breuer et al. 1997). In addition, canonical sorting motifs (i.e., D/EXXLL and YXXφ) within the cytosolic regions of the MPRs have been shown to be recognized by components of the vesicular machinery that dictate the localization and intracellular trafficking of the receptors (see Ghosh et al. (2003) and Bonifacino (2004) for reviews).

Carbohydrate binding properties of the MPRs and location of the three carbohydrate binding sites of the CI-MPR

Soluble acid hydrolases constitute a heterogeneous population of over 60 enzymes that differ in size, oligomeric state, number of *N*-linked oligosaccharides, extent of phosphorylation, and the position of the Man-6-P moiety and its linkage to the penultimate mannose residue in the oligosaccharide chain (Figure 1). In order to understand the physiological rationale for the observation that both MPRs are expressed in most cell types, the relative contribution of each MPR to the targeting of this diverse population of enzymes to the lysosome has been examined. The two MPRs have been shown to display different affinities and capacities for the transport of the various acid hydrolases, and studies using receptor-deficient fibroblasts demonstrate that both receptors are necessary for the efficient sorting of all lysosomal enzymes to the lysosome as neither MPR can fully compensate for the other (Pohlmann et al. 1995; Kasper et al. 1996; Munier-Lehmann et al. 1996; Sleat and Lobel 1997). These studies indicate that the two MPRs recognize distinct but overlapping populations of acid hydrolases. A recent proteomic analysis of serum from mutant mice deficient in either the CD-MPR or CI-MPR revealed that several lysosomal proteins are preferentially sorted by the CD-MPR (e.g., tripeptidyl peptidase I) or CI-MPR (e.g., cathepsin D) (Qian et al. 2008). While the molecular basis for this apparent selective sorting was not investigated, this approach is likely to lead to future studies that will shed light onto the functional significance of two distinct MPRs in a given cell type.

The CD-MPR and CI-MPR share a number of similarities with respect to carbohydrate recognition. For example, both MPRs bind the monosaccharide Man-6-P with essentially the same affinity ($7–8 \times 10^{-6}$ M) (Tong et al. 1989; Tong and Kornfeld 1989). The axial 2-hydroxyl group and the 6-phosphate monoester group are major determinants of binding specificity based on the observation that mannose or glucose 6-phosphate interact poorly with the MPRs ($K_i = 1–5 \times 10^{-2}$ M) (Tong and Kornfeld 1989). A number of synthetic analogs have also been generated and those with the highest affinity to the CI-MPR were found to be isosteric to Man-6-P. Although analogs containing two negative charges were the best ligands, the presence of a phosphorous atom was not necessary for recognition (reviewed in Gary-Bobo et al. (2007)). Inhibition studies using chemically synthesized oligomannosides or neoglycoproteins demonstrated that the presence of the phosphomonoester Man-6-P at a terminal position is the major determinant of receptor binding. In addition, linear mannose sequences which contain a terminal Man-6-P linked α1,2 to the penultimate mannose were shown to be the most potent inhibitors (Distler et al. 1991; Tomoda et al. 1991), suggesting that the MPRs bind an extended oligosaccharide structure which includes the Man-6-P α1,2 Man sequence. Our recent crystal structure of the CD-MPR complexed with an α1,2-linked phosphorylated trimannoside has demonstrated the positioning of the penultimate and prepenultimate mannose rings in the binding pocket and revealed their hydrogen bond interactions with the receptor (Olson et al. 2008). Furthermore, multivalent interactions between the receptor and a lysosomal enzyme result in high affinity binding, typically on the order of 1–10 nM for both MPRs (Sleat and Lobel 1997; Tong and Kornfeld 1989; Watanabe et al. 1990).

In contrast to these similarities, the two MPRs exhibit a number of differences in their binding properties which include pH dependence, cation dependence, and recognition of phosphodiesters. The two MPRs display optimal ligand binding at ~pH 6.4 and no detectable binding below pH 5, which is consistent with their function of releasing ligands in the acidic environment of the endosome. The CI-MPR retains phosphomannosyl binding capabilities at neutral pH which corresponds well with the ability of this receptor to bind and internalize lysosomal enzymes at the cell surface. In contrast, the ligand binding ability of the CD-MPR is dramatically reduced at a pH > 6.4 (Tong et al. 1989; Tong and Kornfeld 1989) which is consistent with its decreased ability to bind and internalize lysosomal enzymes at the cell surface (Stein et al. 1987). The inability to purify the CD-MPR by phosphomannosyl affinity chromatography performed in the absence of cations led to its designation as a "cation-dependent" receptor (Hoflack and Kornfeld 1985). However, the presence of cations increases the binding affinity of the CD-MPR towards Man-6-P (Tong and Kornfeld 1989) and lysosomal enzymes only 4-fold (Sun et al. 2005) but has no effect on the binding affinity of the CI-MPR. This finding differentiates the CD-MPR from C-type lectins which have an absolute requirement for calcium to carry out their sugar binding activities (Drickamer 1999). Mutagenesis studies (Sun et al. 2005) evaluated in the context of the crystal structure (Roberts et al. 1998; Olson, Zhang, et al. 1999) indicate that a conserved aspartic acid residue at position 103 of the CD-MPR, which is not present in the CI-MPR, necessitates the presence of a divalent cation in the

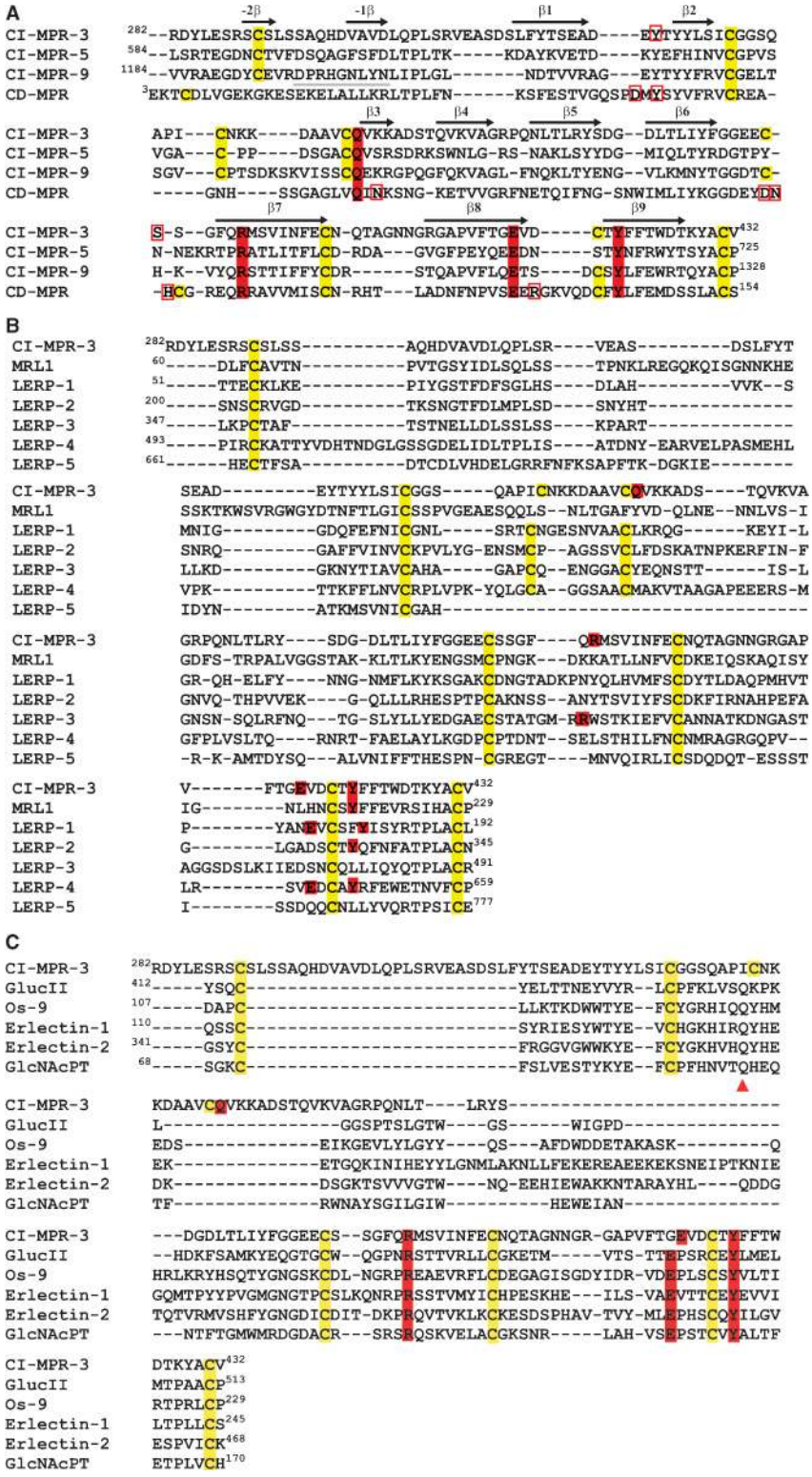

Fig. 3. Sequence alignment of the Man-6-P binding sites of the bovine CI-MPR and bovine CD-MPR. (**A**) Structure-based amino acid sequence alignment of the extracytoplasmic region of the CD-MPR and domains 3, 5, and 9 of the CI-MPR. The secondary structure of domain 3 of the CI-MPR and the CD-MPR are shown, with *arrows* representing the β-strands and the cylinder representing an α-helix. At the N-terminus, domain 3 of the CI-MPR contains two β-strands (−2β and −1β) whereas the CD-MPR contains a single α-helix. The cysteine residues are boxed in *yellow*. Residues that are within hydrogen bonding distance of Man-6-P, as determined by the crystal structure of the CD-MPR (PDB 1C39) and domains 1–3 of the CI-MPR (PDB 1SZO) are boxed in *red*, with the four residues essential for Man-6-P binding (i.e., Gln, Arg, Glu, and Tyr) shaded in *red*. (**B**) Sequence alignment of domain 3 of the bovine CI-MPR with the single MRH domain of *S. cerevisiae* Mrl and the five MRH domains of *Drosophila* LERP. (**C**) Sequence alignment of domain 3 of the bovine CI-MPR with the MRH domains of human glucosidase II β subunit, human OS-9, human XTP3-B/Erlectin, and human GlcNAc-phosphotransferase γ subunit. The *red triangle* indicates the position of the putative conserved Gln. Sequence alignments in panels B and C were performed using M-Coffee (Wallace et al. 2006; Moretti et al. 2007).

binding pocket to obtain high affinity ligand binding by functioning to neutralize the negative charge of Asp103 juxtaposed to the phosphate oxygen of Man-6-P. The CI-MPR, unlike the CD-MPR, is able to recognize Man-P-GlcNAc phosphodiesters (Tong et al. 1989; Tong and Kornfeld 1989; Distler et al. 1991) as well as lysosomal enzymes derived from *Dictyostelium discoideum* which contain mannose 6-sulfate residues and small methyl phosphodiesters, Man-6-P-OCH_3, but not phosphomonoesters (Gabel et al. 1984; Freeze 1986).

The MPRs differ in the number of Man-6-P binding sites contained within their polypeptide chain, with the CD-MPR containing one (Tong and Kornfeld 1989) and the CI-MPR containing three Man-6-P binding sites (Tong et al. 1989; Reddy et al. 2004). Expression of recombinant truncated forms of the CI-MPR has mapped its three carbohydrate binding sites: two high affinity sites ($K_i = \sim 10$ μM for Man-6-P) map to domains 1–3 and domain 9 (Hancock, Yammani, et al. 2002) while domain 5 houses a low affinity ($K_i = \sim 5$ mM for Man-6-P) binding site (Reddy et al. 2004) (Figure 2). A comparison of the binding properties of the individual carbohydrate recognition sites demonstrated that domain 9 of the CI-MPR exhibits optimal binding at pH 6.4–6.5, similar to that of the CD-MPR. In contrast, the N-terminal Man-6-P binding site (i.e., domains 1–3) has a significantly higher optimal binding pH of 6.9–7.0 (Marron-Terada et al. 2000). This observation may not only explain the relatively broad pH range of ligand binding by the CI-MPR but likely is a main contributor to the ability of the CI-MPR, as opposed to the CD-MPR, to internalize exogenous ligands at the slightly alkaline pH 7.4 present at the cell surface. Domain 9 of the CI-MPR, like the CD-MPR, is highly specific for phosphomonoesters (Chavez et al. 2007). In contrast, the N-terminal carbohydrate recognition site of the CI-MPR is promiscuous in that, in addition to Man-6-P, it is able to efficiently bind mannose 6-sulfate and the Man-6-P-OCH_3 phosphodiester with only a 20-fold or 10-fold, respectively, lower affinity than Man-6-P (Marron-Terada et al. 2000). Recent studies using surface plasmon resonance analyses demonstrate that unlike the CD-MPR and domain 9 of the CI-MPR, domain 5 of the CI-MPR exhibits a 14- to 18-fold higher affinity for Man-P-GlcNAc than Man-6-P and implicates this region of the receptor in targeting phosphodiester-containing lysosomal enzymes (i.e., those acid hydrolases that escaped the action of the uncovering enzyme in the TGN) to the lysosome (Chavez et al. 2007). However, additional studies are required to probe whether other glycan structures may be the preferred ligand for this region of the receptor. Taken together, the presence of three distinct carbohydrate recognition sites in the CI-MPR likely accounts for the ability of the CI-MPR to recognize a greater diversity of ligands than the CD-MPR both in vitro (Hoflack et al. 1987; Sleat and Lobel 1997) and in vivo (Ludwig et al. 1994; Pohlmann et al. 1995; Kasper et al. 1996; Sohar et al. 1998).

The multifunctional CI-MPR interacts with several non-Man-6-P-containing ligands

The CI-MPR has been shown to interact with a number of molecules on the cell surface in a Man-6-P-independent fashion. These ligands include insulin-like growth factor II (IGF-II) (Dahms et al. 1994; Schmidt et al. 1995), urokinase-type plasminogen activator receptor (uPAR) (Nykjaer et al. 1998), plasminogen (Godar et al. 1999), retinoic acid (Kang et al. 1997), serglycin (Lemansky et al. 2007), and heparanase (Wood and Hulett 2008). Limited information is available concerning the receptor's interaction with retinoic acid. However, photolabeling studies of the full-length (300 kDa) and truncated serum form (∼260 kDa) of the CI-MPR with [^{3}H] retinoic acid indicated that the ∼40 kDa C-terminal region of the receptor, which is absent in the serum form of the CI-MPR, is essential for retinoic acid binding (Kang et al. 1997). In addition, the nature of the interaction between the CI-MPR and uPAR is unclear as a recent report which utilized coimmunoprecipitation assays indicated that the serum form of the CI-MPR binds uPAR in a Man-6-P-dependent fashion whereas the membrane-associated full-length CI-MPR binds uPAR in a Man-6-P-independent manner (Kreiling et al. 2003). Of these non-Man-6-P-containing ligands, the interaction between the CI-MPR and IGF-II has been studied most extensively, and in the literature the CI-MPR is also referred to as the IGF-II receptor. The IGF-II binding site has been mapped to domain 11, with residues in domain 13 increasing the affinity of the interaction by ∼10-fold (Devi et al. 1998; Linnell et al. 2001). The uPAR and plasminogen binding sites have been localized to domain 1 (Leksa et al. 2002) (Figure 2). Although the interactions between the receptor and these ligands are protein–protein or protein–lipid mediated, the interaction between the CI-MPR and serglycin, a lysosomal soluble proteoglycan, appears to be carbohydrate-based via serglycin's chondroitin sulfate chains (Lemansky et al. 2007). Based on our observation that domains 1–3, but not domain 9, of the CI-MPR can interact with Man-6-sulfate (Marron-Terada et al. 2000), we predict that serglycin binds to the N-terminal region (i.e., domains 1–3) of the receptor. Additional studies will be needed to test this hypothesis and to evaluate fully the carbohydrate specificity of the three different binding sites of the CI-MPR.

Crystal structure of truncated forms of the CD-MPR and CI-MPR

The extracytoplasmic region of the CD-MPR has been studied extensively, with crystal structures of this receptor obtained bound to a single sugar (Man-6-P (Roberts et al. 1998)), or to various oligosaccharide structures (pentamannosyl phosphate (Olson, Zhang, et al. 1999)), an α1,2-linked phosphorylated trimannoside, or a nonphosphorylated branched oligosaccharide Man(α1,3)(Man(α1,6))Man(β1,4)GlcNAc(β1,4)GlcNAc (Olson et al. 2008). The CD-MPR has also been crystallized under different pH conditions: in an unbound state at pH 6.5 (Olson et al. 2002) and pH 4.8 (Olson et al. 2008), or at pH 7.4 in a bound state (Olson et al. 2008). Seven out of the fifteen domains of the CI-MPR have also been crystallized: the N-terminal 432 residues (domains 1, 2, and 3), which house a high affinity Man-6-P binding site, bound to either a mannose residue from a crystallographic neighbor (Olson, Yammani, et al. 2004) or Man-6-P (Olson, Dahms, et al. 2004); the IGF-II binding site (domains 11–14) in the unbound state (Brown et al. 2002; Uson et al. 2003) or domains 11–13 in an IGF-II bound state (Brown et al. 2008). These structures have provided a framework from which the mechanism of ligand binding by the MPRs can be inferred.

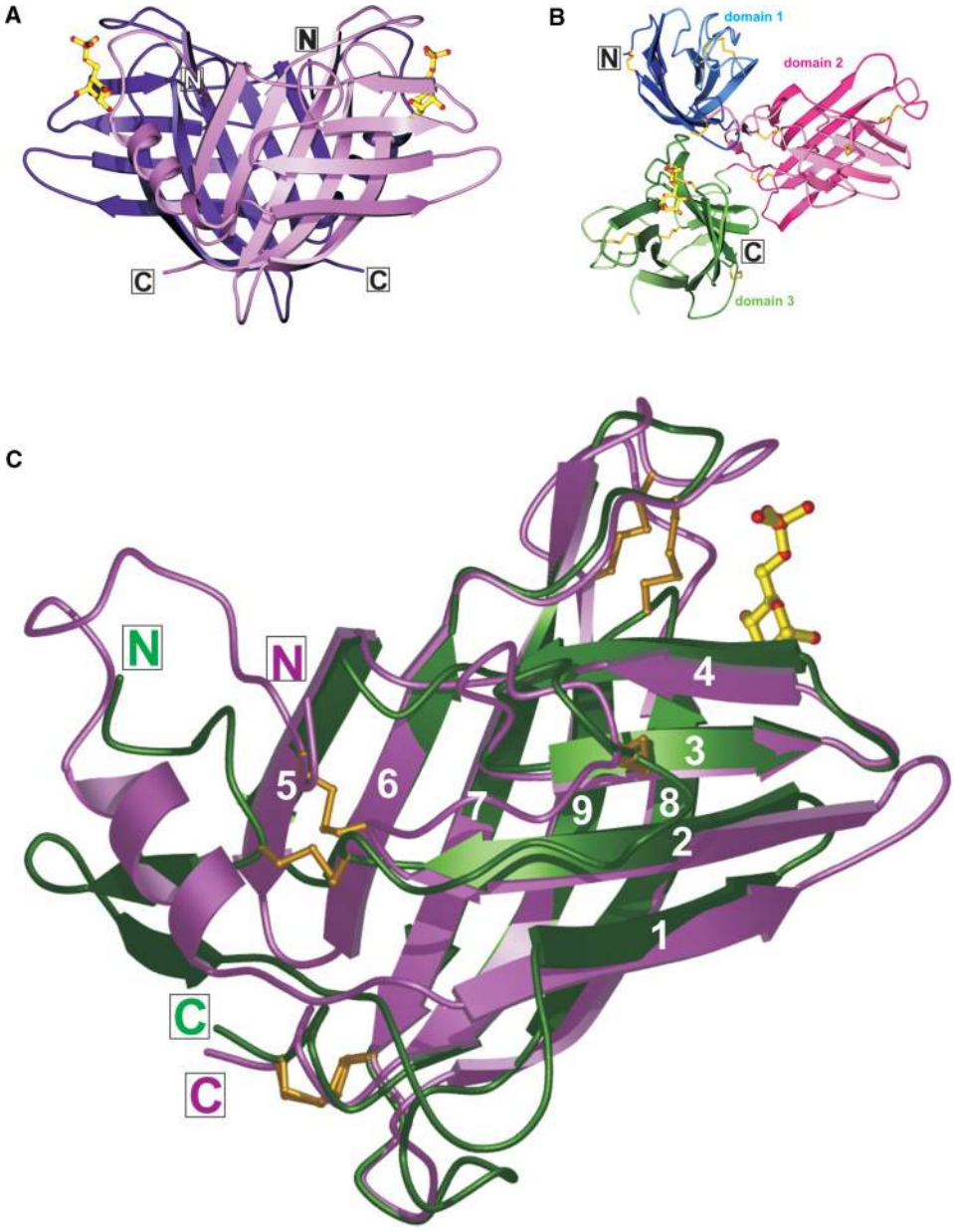

Fig. 4. Three-dimensional structure of the CD-MPR and domains 1–3 of the CI-MPR. (**A**) Crystal structure of the extracytoplasmic region (residues 3–154) of the bovine CD-MPR in the presence of an oligosaccharide, pentamannosyl phosphate (PDB 1C39). Note that only the terminal Man-6-P (*gold* ball-and-stick model) is shown for clarity. Both monomers (*light purple* and *dark purple*) of the CD-MPR dimer are shown in this ribbon diagram. The N-terminus (N) and C-terminus (C) are boxed. (**B**) Crystal structure of the N-terminal three domains (residues 7–432) of the bovine CI-MPR (PDB 1SZO). The N- and C-terminus of the protein encoding domain 1 (*blue*), domain 2 (*pink*), and domain 3 (*green*) are indicated. The location of Man-6-P (*gold* ball-and-stick model) is shown. (**C**) Overlay of the structures of the CD-MPR (*purple*) and domain 3 (*green*) of the CI-MPR. The β-strands are sequentially numbered. The location of Man-6-P in the binding pocket (*gold* ball-and-stick model) is shown. The disulfide bridges are shown in *gold*, and the N- and C-terminus are boxed.

The extracytoplasmic domain of the CD-MPR crystallized as a dimer (Figure 4A), which is consistent with previous biochemical data. Each polypeptide chain folds into an N-terminal α-helix followed by four anti-parallel β-strands which together comprise the solvent-exposed front face. The dimer interface β-sheet (β5–β9), which accounts for approximately 20% of the surface area of the monomer, is composed of five anti-parallel β-strands, with strand 9 interjecting between strands 7 and 8 (Figure 4C). Subsequent determination of the structure of domains 1–3 (Figure 4B) and domains 11–14 of the CI-MPR showed that this overall topology is conserved with the exception of the N-terminal region: neither domains 1–3 nor 11–14 contain the α-helix; rather, this secondary structural element is replaced by two β-strands (Figure 3). The quaternary domain arrangement of CD-MPR is not conserved in the structure of domains 1–3 of the CI-MPR. The structure of the N-terminal region of the CI-MPR shows the three domains form a wedge with domains 1 and 2 oriented such that the four-stranded N-terminal sheet (β1–β4) of domain 1 and the five-stranded C-terminal sheet (β5–β9) of domain 2 form a continuous

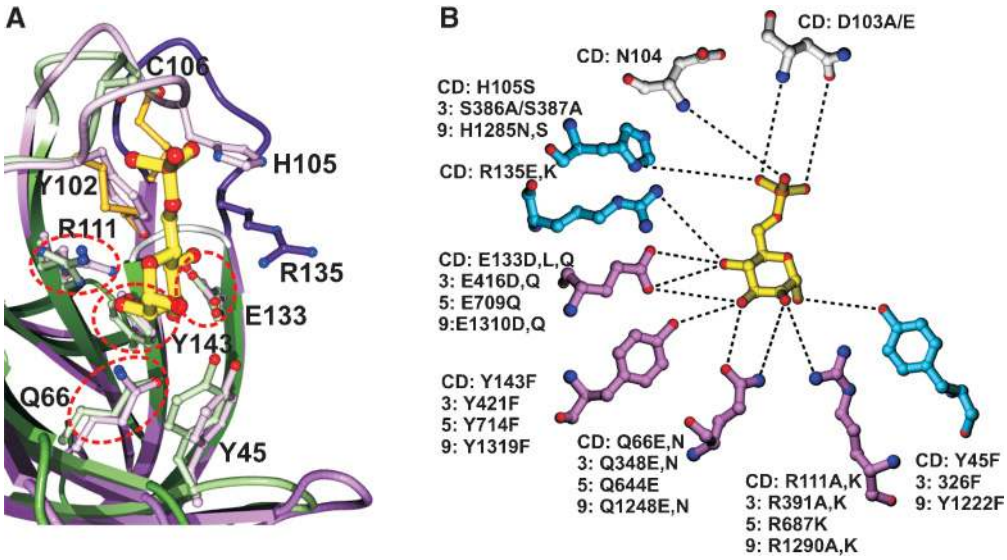

Fig. 5. Comparison of the carbohydrate binding pocket of the CD-MPR and domain 3 of the CI-MPR. (**A**) Ribbon diagram showing the binding site of the CD-MPR (*purple*) superimposed onto domain 3 (*green*) of the CI-MPR. The disulfides are shown in *gold* and Man-6-P (*gold* ball-and-stick model) is also indicated. The four residues that are essential for Man-6-P binding (i.e., when replaced result in an ∼1,000 reduction in affinity) are circled in *red*. H105 is located within loop C. R135 is located within the relatively long loop D (*dark purple*) in the CD-MPR structure. In contrast, loop D (*light gray*) is short in domain 3. (**B**) Schematic view of the potential hydrogen bond and ionic interactions between the binding pocket residues of the CD-MPR and Man-6-P (*gold* ball-and-stick). The binding pocket residues and the single amino acid substitutions that were made are listed for the CD-MPR and domain 3, domain 5, and domain 9 of the CI-MPR. Shaded in *light gray* are the residues that have not been tested (N104) or when mutated (D103) retained wild-type Man-6-P binding ability. Shaded in *blue* are the residues that when mutated resulted in receptors with diminished (∼50–150-fold) Man-6-P binding ability as compared to wild-type receptors. Shaded in *purple* are the residues identified as essential for carbohydrate recognition by the MPRs (i.e., single amino acid substitution of these residues abolished (∼1000-fold) the Man-6-P binding ability of the receptors) (Wendland, Waheed, et al. 1991; Olson, Hancock, et al. 1999; Hancock, Haskins, et al. 2002; Sun et al. 2005; Chavez et al. 2007).

surface (Figure 4B). In comparison to the CD-MPR in which extensive contacts exist between the two dimer interface β-sheets (β5–β9) (Figure 4A), the interaction between the three N-terminal domains of the CI-MPR is quite different and much less extensive; the contacts between the three domains are mediated mainly by residues within the linker regions and loops (Figure 4B). However, the contacts between domains 1, 2, and 3 are important for maintaining the integrity of the binding pocket housed within domain 3. The multiple interactions between residues of domains 1 and 2 with residues of loops C and D of domain 3 are likely to aid in the stabilization of the binding pocket and provide an explanation for the inability of a construct encoding domain 3 alone to generate a high affinity carbohydrate binding site (Hancock, Yammani, et al. 2002).

A comparison of the sugar binding pocket of the CD-MPR and domain 3 of the CI-MPR reveals that residues which interact with the mannose ring (Gln, Arg, Glu, and Tyr) are located in a strikingly similar position in the (base[@] ofthe pocket (Figure 5A) and form the same contacts with the ligand (Figure 5A and B). These four residues have been shown to be essential for Man-6-P recognition by mutagenesis studies (Figure 5B) and are conserved in all species and in the other two Man-6-P binding sites of the CI-MPR (i.e., domains 5 and 9) (Figure 3A). The presence of this (signature motif[@] for phosphomannosyl binding (Gln, Arg, Glu and Tyr) along with conserved cysteine residues (Figure 3A) allowed for the prediction that domain 5 of the CI-MPR contains a Man-6-P binding site, a hypothesis which was subsequently confirmed (Reddy et al. 2004). Furthermore, mutation of Gln, Arg, Glu, and Tyr in domains 5 (Chavez et al. 2007) and 9 (Hancock, Haskins, et al. 2002) demonstrates their essential role in carbohydrate recognition by these binding sites (Figure 5B). Thus, the strict requirement for a terminal mannose residue by both receptors is reflected in the similarities in that region of the binding pocket responsible for sugar recognition.

In contrast, the phosphate recognition region (lid[@]) of the binding site appears to have the most variability both in amino acid composition and in structure. In both receptors the lid is formed by residues joining β-strands 6 and 7 (loop C). This lid region is larger in the CD-MPR and the positioning of the disulfide anchors the loop in a more closed position which translates into a more sterically confined binding region (Figure 5A). The conformationally constrained lid may account for the inability of CD-MPR to bind phosphodiesters. Shortening of both loops C and D effectively makes the binding pocket of domains 1–3 more open than that of the CD-MPR (Figure 5A), allowing for this region of the CI-MPR to bind a larger repertoire of ligands, including phosphomonoesters, mannose 6-sulfate, and phosphodiesters. Thus, the diversity in ligand recognition by the two receptors appears to be accomplished by alterations in the receptor binding site architecture surrounding the phosphate moiety.

CD-MPR is dynamic and adopts at least two different conformations

The MPRs encounter a variety of conditions as they travel to various compartments where they bind and release their ligands. Key to their function is the pH-dependence of ligand interaction. Cells treated with reagents that raise the pH of endosomal/lysosomal compartments exhibit decreased sorting

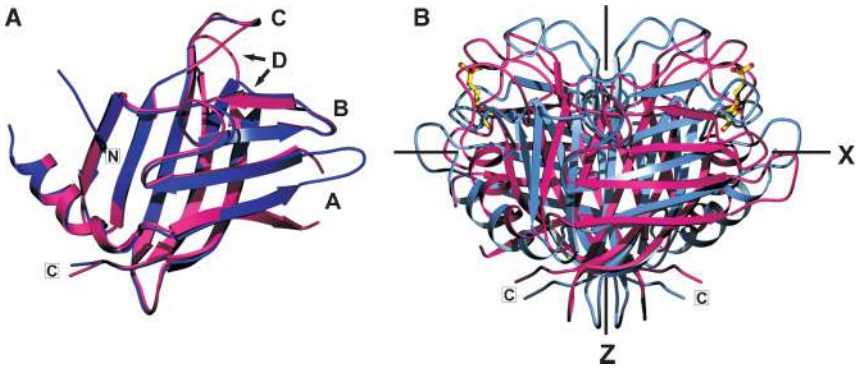

Fig. 6. Comparison of the bound and unbound conformations of the CD-MPR. (**A**) Superimposition of the monomers of the bound (PDB 2RL8, *red*) and unbound (PDB 2RL7, *blue*) forms of the CD-MPR. The location of loop D, which exhibits the most dramatic change in position, is indicated. Loops A, B, and C are also labeled. The C- and N-termini are boxed. (**B**) Superimposition of all Cα atoms of the dimer of the bound (PDB 2RL8, *red*) and unbound (PDB 2RL7, *blue*) structures. Between the two conformations, there is an ∼30° scissoring motion between the two subunits of the dimer with respect to the two-fold axis (*Z* axis) on the *XZ* plane and an ∼12° twist along the *Z*-axis pivoting at the center of the dimer molecule. The C- and N-termini are boxed. The location of Man-6-P in the binding pocket (*gold* ball-and-stick model) is shown.

of lysosomal enzymes to lysosomes and a concomitant increase in the secretion of these enzymes into the medium (Imort et al. 1983). This observation implies that it is essential for the MPRs to release their ligands in the acidic environment of endosomes in order to be able to recycle back to the TGN to retrieve additional lysosomal enzymes. To determine whether different pH conditions elicit conformational changes in the receptor that alters ligand binding affinities, we have obtained numerous crystal structures of the CD-MPR under conditions representative of the various environments encountered by the receptor: bound state at pH 6.5 and pH 7.4 and unbound state at pH 6.5 and pH 4.8 (Olson et al. 2002, 2008). Surprisingly, all ten structures of the CD-MPR obtained to date can be categorized into one of two conformations: an "open" conformation found in all structures containing ligand in the binding pocket and a "closed" conformation found in all structures missing bound carbohydrate (Figure 6). Unlike what has been observed in other lectins, the structure of the ligand-free CD-MPR differs considerably from the ligand-bound form in that changes in both quaternary structure and positioning of loops involved in sugar binding are seen, along with changes in the spacing of the two carbohydrate binding sites in the dimeric receptor (the Cα atoms of His105 located in Loop C are ∼34 Å apart in the open conformation and ∼26 Å apart in the closed conformation). Loop D (residues Glu134–Cys141) exhibits the most dramatic change in position, with Val138 displaying the largest displacement (Cα–Cα distance of 16 Å) (Figure 6A). The CD-MPR differs dramatically from other lectins in an unbound state, where water molecules fill the shallow binding grooves of other most lectins in the absence of bound sugar. Instead of essential side chain interactions being shifted from the carbohydrate hyroxyls to water, the pocket of the CD-MPR undergoes restructuring: loop D swings into the binding pocket in the absence of ligand and provides contacts that hold essential residues in the proper orientation so that they are maintained in a "ready-state" to accept ligand. The two conformations also display a dramatic difference in their quaternary structure that can be described globally as a scissoring and twisting motion between the two subunits of the dimer (Figure 6B). Taken together, these results indicate that the CD-MPR is dynamic and must be able to transition between these two conformations as it moves to different organelles, with the unique environment of each organelle impacting the equilibrium between the two states.

Based on analyses of these structures, distinct mechanisms for the dissociation of lysosomal enzymes at the cell surface and under the acidic conditions of the endosome were proposed for the CD-MPR (Olson et al. 2008). His105 is the only residue of the receptor in which a titratable side chain is involved in binding the phosphate group of Man-6-P (Figure 5A). Deprotonation of His105 and the phosphate moiety of Man-6-P appear to be key elements in the release of ligand at the cell surface: loss of the electrostatic interaction between the now uncharged His105 and Man-6-P is predicted to facilitate dissociation of phosphorylated ligands at pH 7.4. In the acidic environment of the endosome, it is proposed that disruption, via protonation, of intermonomer electrostatic interactions that tie loop D of one monomer to the α-helix of the other monomer in the ligand bound conformation would "free" loop D to move into the binding pocket, resulting in the displacement of ligand. In addition, protonation of Glu133 that is located in the binding pocket (Figure 5A) is predicted to weaken its interaction with the 3- or 4-hydroxyl group of Man-6-P (Figure 5B) and disrupt the electrostatic environment of the entire binding pocket, thereby enhancing the release of Man-6-P. The repositioning of loop D into the binding pocket eliminates its intermonomer interaction with the N-terminal α-helix. This loss of intermonomer contact may trigger the reorientation of the two monomers as the receptor changes its quaternary structure, adopting a more closed conformation in the unbound state.

Orientation of the 15 domains in the extracellular region of the CI-MPR

How are the ligand binding sites within the large extracellular region of the CI-MPR arranged? This question has been partially answered since the crystal structures of one of the Man-6-P binding sites (domains 1–3) (Olson, Dahms, et al. 2004; Olson, Yammani, et al. 2004) and the IGF-II binding site (domains 11–14) (Brown et al. 2008) have been obtained.

The structure of domains 1–3 is very compact and forms a triangular disk of 70 Å (each side) × 50 Å (thickness), with each corner of the triangle occupying one domain. In contrast, the relative orientations of domains 11–14 are very different. The structure of domains 11–14 is rather elongated (50 Å × 60 Å × 115 Å high) and resembles beads on a string, with each domain forming a bead. Combining these two modular structures, along with the prediction that the remaining two Man-6-P binding sites will each occur in a tri-domain compact structure like the architecture of domains 1–3, a simplified representation of the entire extracellular portion (domains 1–15) of the CI-MPR is shown in Figure 2. A similar model has been proposed by Brown et al. (2008). Consistent with the three-domain architecture for each Man-6-P binding site is the presence of a proteolytically sensitive site between domains 6 and 7 (Westlund et al. 1991). The structure of the IGF-II binding site reveals that IGF-II binds in the same location as Man-6-P, thus the same MRH fold can function in protein–protein or protein–carbohydrate interactions. What is not clear is how the various ligand binding sites are oriented relative to each other and whether the CI-MPR undergoes conformational changes that may be influenced by pH or ligand binding. Although it is intriguing that all ligand binding sites have been mapped to odd numbered domains, there are no data to support the positioning of these binding sites on one face of the molecule as we have modeled here (Figure 2). Answers to these questions await further structural studies.

Evolution of the MPRs and members of the MRH protein family

Expression of the MPRs

The Man-6-P-based system for targeting lysosomal hydrolases to lysosomes is conserved in mammals, birds, amphibians, and crustaceans but is absent in the unicellular protozoa *Trypanosoma* (Huete-Perez et al. 1999) and *Leishmania* (Clayton et al. 1995). *D. discoideum* and *Acanthamoeba castellani* both exhibit GlcNAc-phosphotransferase activity and can transfer GlcNAc-1-PO4 to mannose residues (Lang et al. 1986). However, MPRs have not been identified in these species. The recently reported sequence of the zebrafish (*Danio rerio*) CD-MPR and CI-MPR (Nolan et al. 2006) indicates that targeting of lysosomal enzymes by MPRs represents an ancient pathway in vertebrate cell biology. The CD-MPR (Nadimpalli and von Figura 2002) and CI-MPR (Lakshmi et al. 1999; Nadimpalli et al. 2004) have also been reported in the invertebrate mollusc *Unio*. Taken together, these studies and others demonstrate that numerous species throughout the animal kingdom express *bone fide* MPRs that are capable of binding phosphomannosyl residues.

In recent years, several other proteins (Mrl1, LERP, GlcNAc-1-phosphotransferase γ-subunit, ER glucosidase II β-subunit, OS-9, and XTP3-B/Erlectin) have been identified that contain MRH domains (Dodd and Drickamer 2001; Munro 2001; Cruciat et al. 2006) (Figure 2), but to date none have been shown to bind Man-6-P. Of these, only LERP has been shown to function in the delivery of lysosomal enzymes to the lysosome (see below). In yeast, the mechanism by which soluble hydrolases, such as carboxypeptidase Y and proteinase A, reach the vacuole (functional equivalent of the lysosome) is very similar to that found in mammalian cells except that the vacuolar sorting signal is not carbohydrate-based, but rather resides in the propeptide region of the hydrolase and is recognized by a receptor, Vps10, that cycles between the Golgi and endosomal compartments (Ni et al. 2006). Whyte and Munro (2001) identified a yeast protein, Mrl1, that is predicted to be a type I membrane glycoprotein and contains a single MRH domain like the CD-MPR. Mrl1 was shown to colocalize with Vps10 in *Saccharomyces cerevisiae*. No significant effect on the delivery of carboxypeptidase Y or proteinase A was observed in *S. cerevisiae* strains lacking MRL1. However, deletion of both MRL1 and VPS10, in contrast to VPS10 alone, had a marked effect on the sorting of proteinase A to the vacuole. Thus, the possibility exits that Mrl1 and Vps10 may function as coreceptors in the sorting of proteinase A to the vacuole. Analysis of the sorting of proteinase A in an Alg3 deletion strain (synthesizes shorter *N*-glycans that are enriched in Man5 structures) indicated that a specific *N*-glycan structure is not required for Mrl1 activity. The observation that Mrl1 contains only one of the conserved residues involved in Man-6-P recognition (Figure 3B) suggests that Mrl1 is an unlikely candidate for functioning as a mannose-specific lectin. Clearly, additional studies are required to determine the role, whether direct or indirect, Mrl1 may have on the targeting of vacuolar hydrolases.

Drosophila lysosomal enzyme receptor protein (LERP) targets lysosomal enzymes in a Man-6-P-independent fashion

The existence of a Man-6-P-dependent transport pathway for lysosomal enzymes in insects has been unclear since Man-6-P has been reported on a lysosomal enzyme (DNase I) derived from *Drosophila melanogaster* (Gaszner and Udvardy 1991) whereas Man-6-P-containing oligosaccharides or GlcNAc-phosphotransferase activity was not detected in the Sf9 insect cell line (Aeed and Elhammer 1994). Recently, a *Drosophila* protein (LERP) that is related to the mammalian CI-MPR has been identified (Dennes et al. 2005). The amino acid sequence predicts a type I membrane glycoprotein containing five contiguous MRH domains, each about 155 residues in length, in its luminal region that the authors propose correspond to domains 9–13 of the CI-MPR (Figure 2). The authors showed that LERP, which is able to interact with *Drosophila* and mammalian Golgi-localized, Gamma-ear-containing, ADP-ribosylation factor-binding (GGA) adaptors that have been shown to sort MPRs in clathrin-coated vesicles at the TGN (Bonifacino 2004; Ghosh and Kornfeld 2004), mediates lysosomal enzyme targeting and rescues the missorting of lysosomal enzymes that occurs in MPR-deficient mammalian cells. Although the nature of the interaction between *Drosophila* LERP and mammalian lysosomal enzymes has not yet been elucidated, it does not involve Man-6-P since no detectable binding was observed between LERP and a Man-6-P-containing affinity resin (phosphomannan) and the sorting of mammalian lysosomal enzymes by LERP was not inhibited by the addition of Man-6-P to the medium. Furthermore, the four residues that are essential for Man-6-P recognition are not conserved in the *Drosophila* protein, with only domains 1 and 4 containing possibly two out of the four residues (Figure 3B). It is intriguing to speculate that LERP may represent the evolutionary intermediate between yeast and the animal kingdom: *Drosophila* LERP acquired

multiple copies of MRH domains, but the MRH domains do not yet have the ability to bind carbohydrate.

Resident soluble ER (glucosidase II β-subunit, OS-9, and XTP3-B/Erlectin) and Golgi (GlcNAc-phosphotransferase γ-subunit) proteins containing MRH domains are implicated in N-glycan recognition

In contrast to the membrane proteins CD-MPR, CI-MPR, Mrl1, and LERP, the other MRH domain-containing proteins are soluble proteins found in the ER and Golgi (Figure 2). The β-subunit of glucosidase II contains a single MRH domain plus a C-terminal ER retention signal (HDEL) and is the noncatalytic subunit of the dimeric ER-resident enzyme involved in the processing of *N*-glycans on nascent glycoproteins (Munro 2001). OS-9 contains a single MRH domain and was originally identified as a protein upregulated in human osteosarcomas (Kimura et al. 1998). Recent studies on the Yos9 protein, the *S. cerevisiae* homolog of OS-9 which, in contrast to the human OS-9, contains a C-terminal ER retention signal (HDEL), indicate that this ER-resident protein plays an essential role in the recognition of misfolded glycoproteins during ER-associated degradation (ERAD) (Bhamidipati et al. 2005; Kim et al. 2005; Szathmary et al. 2005). However, its role as a lectin is controversial as Yos9 is able to bind misfolded proteins lacking any glycans (Bhamidipati et al. 2005). Evidence for its ability to recognize carbohydrate is indirect as mutation of the putative sugar binding residues in its MRH domain abolishes its function in the degradation of misfolded proteins (Bhamidipati et al. 2005; Szathmary et al. 2005). XTP3-B, also referred to as Erlectin, is a luminal ER-resident protein first characterized in Xenopus that appears to function as an ER chaperone for Krm2, a coreceptor for Dkk1 which plays a role in head induction during early Xenopus development (Cruciat et al. 2006). XTP3-B/Erlectin contains two MRH domains, and MRH domain 2, but not MRH domain 1, of this protein mediates interaction with Krm2. The finding that enzymatic deglycosylation of Krm2 abolishes its interaction with XTP-3/Erlectin in vitro further implicates XTP3-B/Erlectin as a lectin. In support of the above studies, a recent report by Christianson et al. (2008) showed that human OS-9 and human XTP3-B/Erlectin interact with distinct sets of ERAD substrates; the authors propose that the MRH domains of these proteins contribute to the interaction with other components of the ERAD quality control machinery rather than with ERAD substrates directly. In these studies, mutation of the putative sugar binding residues in XTP3-B/Erlectin MRH domain 1 and/or MRH domain 2 inhibited the interaction of XTP3-B/Erlectin with the Hrd1-SEL1L ubiquitin ligase complex involved in degradation of ERAD substrates, indicating that both MRH domains of XTP3-B/Erlectin are important for its function. In contrast to the findings of Christianson et al., mutation of a putative sugar binding residue in the MRH domain of OS-9 does not affect its substrate binding activity (Bernasconi et al. 2008). Taken together, a clear picture has not yet emerged concerning the role of the MRH domains in Yos9, OS-9, and XTP3-B/Erlectin.

An intriguing observation is that both essential components of the lysosomal enzyme targeting machinery, the MPRs and the GlcNAc-phosphotransferase, contain MRH domains. The γ-subunit of GlcNAc-phosphotransferase, which is the noncatalytic subunit of the $\alpha_2\beta_2\gamma_2$ hexameric complex involved in generating the Man-6-P tag on lysosomal enzymes, contains a single MRH domain (Munro 2001) (Figure 2). A recent study by Kornfeld and co-workers (Lee et al. 2007) has shown that transgenic mice deficient in the γ-subunit of the GlcNAc-phosphotransferase still retain substantial activity (i.e., posttranslational modification with GlcNAc-P) toward acid hydrolases. These studies indicate that the γ-subunit is not essential for substrate recognition and that the α/β-subunits, in addition to their catalytic function, have some ability to recognize acid hydrolases as specific substrates. The authors suggest that this specific recognition is somehow enhanced by the presence of the γ-subunit.

Clearly lacking in all of the above studies is a direct, detailed analysis of the putative carbohydrate binding properties of these MRH-containing proteins. However, based on the alignment (Figure 3C) which predicts that their MRH domains contain three out of the four conserved residues (Arg, Glu, Tyr) of the MPRs shown to interact with the 2-, 3-, and 4-hydroxyl groups of the mannose ring of Man-6-P (Roberts et al. 1998; Olson, Dahms, et al. 2004), it is likely that glucosidase II β-subunit, OS-9, XTP3-B/Erlectin, and GlcNAc-phosphotransferase γ-subunit bind specifically to high mannose-type oligosaccharides, which would be consistent with their proposed functions in the ER and early Golgi compartments. The alignment (Figure 3C) also shows that the C-terminal back β-sheet (β5–β9) is conserved among these MRH domain containing proteins, which would allow for the correct positioning of three out of the four conserved sugar binding residues within the binding pocket. In contrast, the N-terminal front β-sheet (β1–β4) differs considerably among the proteins, but appears to contain the fourth essential sugar binding residue (i.e., Gln) (Figure 3C). These differences may be due to differences in the lengths of the loops between β-strands, as is observed in the crystal structures of domains 1–3 (Olson, Yammani, et al. 2004) and domains 11–14 of the CI-MPR (Brown et al. 2008). One functional consequence of the nonconserved nature of the N-terminal half of these proteins could relate to protein–protein interactions: the N-terminal front β-sheet may mediate interactions with adjacent subunits (i.e., glucosidase II β-subunit and GlcNAc-phosphotransferase γ-subunit) or with various components of the ERAD machinery (i.e., OS-9 and XTP3-B/Erlectin), with the specificity of the interaction defined by the unique sequences present in the loop regions of the protein.

Bernasconi et al. (2008) suggested that the presence of a single *N*-linked oligosaccharide in the middle of the MRH domain of OS-9 would inhibit the ability of the MRH domain to function in stabilizing a multiprotein complex. However, the CD-MPR contains four *N*-glycans, and domains 3 and 9 of the CI-MPR each contain two *N*-glycans: these MRH domains of the MPRs maintain high affinity Man-6-P binding activity despite the presence of multiple oligosaccharide chains. Both OS-9 and the MRH domain 1 of XTP3-B/Erlectin contain a single *N*-glycan chain at residue 177 (NGS) and 195 (NGT), respectively, which are located in the same position in the alignment (Figure 3C). The position of these *N*-glycans is not conserved with the positions of the *N*-glycans found in the CD-MPR and domains 3 and 9 of the CI-MPR. If the MRH domains of OS-9 and XTP3-B/Erlectin adopt the same fold as the MPRs, Asn177 of OS-9, and Asn195 of XTP3-B/Erlectin are predicted to be located in loop C which forms the top of the carbohydrate binding pocket (i.e.,

located three residues N-terminal to His105 of the CD-MPR, see Figure 5A). Thus, additional studies are needed to test the hypothesis that the presence of an *N*-glycan chain inhibits the lectin activity of OS-9 and/or XTP3-B/Erlectin.

Concluding remarks

Cell biological, biochemical, and biophysical studies have made significant contributions to our understanding of the molecular basis governing the intracellular transport of the MPRs and their mode of carbohydrate recognition. However, many important questions remain unanswered. All of the functional domains of the CI-MPR have been mapped to odd numbered domains (e.g., domain 1, plasminogen/uPAR; domains 3, 5, and 9, Man-6-P; domain 11, IGF-II). However, the role of the remaining 10 unassigned domains is unclear, as is the manner in which the MPRs acquired Man-6-P binding capabilities during evolution. The presence of an MRH domain in the γ-subunit of the GlcNAc-phosphotransferase raises the intriguing possibility that the proteins involved in the synthesis and recognition of Man-6-P evolved together from a common ancestor. Detailed analyses of the structures and putative sugar-binding properties of the MRH domain-containing proteins Mrl1, LERP, glucosidase II β-subunit, OS-9, XTP3-B/Erlectin, and GlcNAc-phosphotransferase γ-subunit will be needed to determine the functional significance of the MRH domains in these proteins. The observation that domain 9 can be expressed as an individual domain, retaining high affinity binding capabilities (Hancock, Yammani, et al. 2002), indicates its mechanism of maintaining and stabilizing its binding pocket must be fundamentally different from that used by domains 1–3 of the CI-MPR. Therefore, structural studies of the CI-MPR to determine the mechanism of carbohydrate binding by domains 5 and 9, the arrangement of all 15 domains within its extracellular region and their relative degree of flexibility, and its mode of oligomerization are essential in order to understand how this large receptor functions to target a diverse array of ligands to endosomal/lysosomal compartments.

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Conflict of interest statement

None declared.

Abbreviations

CD-MPR, cation-dependent mannose 6-phosphate receptor; CI-MPR, cation-independent mannose 6-phosphate receptor; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GGA, Golgi-localizing, γ-ear-containing, ARF-binding protein; GlcNAc-phosphotransferase, UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase; IGF-II, insulin-like growth factor II; LERP, lysosomal enzyme receptor protein; Man-6-P, mannose 6-phosphate; MRH, mannose 6-phosphate receptor homology; TGN, *trans*-Golgi network; uPAR, urokinase-type plasminogen activator receptor.

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