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Carbohydrate Recognition by the Mannose 6-phosphate Receptors

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Summary

The two P-type lectins, the 46 kDa cation-dependent mannose 6-phosphate (Man-6-P) receptor (CD-MPR) and the 300 kDa cation-independent Man-6-P receptor (CI-MPR), are the founding members of the growing family of mannose 6-phosphate receptor homology (MRH) proteins. A major cellular function of the MPRs is to transport Man-6-P-containing acid hydrolases from the Golgi to endosomal/lysosomal compartments. Recent advances in the structural analyses of both CD-MPR and CI-MPR have revealed the structural basis for phosphomannosyl recognition by these receptors and provided insights into how the receptors load and unload their cargo. A surprising finding is that the CD-MPR is dynamic, with at least two stable quaternary states, the open (ligand bound) and closed (ligand free) conformations, similar to those of hemoglobin. Ligand binding stabilizes the open conformation; changes in the pH of the environment at the cell surface and in endosomal compartments weaken the ligand-receptor interaction and/or weaken the electrostatic interactions at the subunit interface, resulting in the closed conformation.

Introduction

In eukaryotic cells, mannose 6-phosphate receptors (MPRs) mediate the delivery of ~60 different newly synthesized soluble acid hydrolases to the lysosome by binding to mannose 6-phosphate (Man-6-P) residues found on their N-linked oligosaccharides. Lysosomal enzymes become differentiated from other proteins in the secretory pathway by acquiring mannose 6-phosphate (Man-6-P) residues in a two step process: 1) The GlcNAc-phosphotransferase transfers N-acetylglucosamine 1-phosphate to one or two mannose residues on an N-glycan to yield a phosphodiester intermediate [1,2]. 2) α-N-acetylglucosaminidase removes the N-acetylglucosamine residue in the trans Golgi network (TGN) to generate the phosphomonoester, Man-6-P [3] (Figure 1). The resulting MPR/lysosomal enzyme complex is transported from the TGN to the late endosome where the low pH of the compartment induces the complex to dissociate. The released enzymes are packaged into lysosomes [4,5] and the receptors either return to the Golgi to repeat the process or move to the plasma membrane where they function to internalize exogenous ligands (Figure 1). The importance of this phosphomannosyl recognition system in the biogenesis of lysosomes is illustrated by the existence of over 40 different human lysosomal storage diseases that are estimated to affect 1 in 5,000 live births [6]. The discovery of the MPRs originated from studies centered on determining the molecular basis of a lysosomal storage disorder, mucolipidosis II (MLII; also

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referred to as “I-cell disease”). The pioneering work of Hickman and Neufeld [7] led to the finding that fibroblasts from MLII patients were capable of internalizing lysosomal enzymes secreted by normal cells, while in contrast, normal fibroblasts were unable to endocytose lysosomal enzymes secreted by MLII fibroblasts. Their hypothesis that lysosomal enzymes contained a recognition tag required for receptor-mediated uptake and transport to lysosomes was later confirmed upon the identification of the tag as Man-6-P [8–10]. I-cell disease is an autosomal recessive disorder caused by a deficiency of GlcNAc-phosphotransferase activity (IUBMB accession number EC 2.7.8.17), which is the enzyme that generates the Man-6-P tag [11] (see Figure 1, Step 1).

Two distinct MPRs, the 46 kDa cation-dependent mannose 6-phosphate receptor (CD-MPR) and the ~300 kDa cation-independent mannose 6-phosphate receptor (CI-MPR) are the sole members of the P-type lectin family [12]. The CI-MPR is multifunctional in that it binds proteins bearing the Man-6-P recognition marker as well as the peptide hormone IGF-II (hence it is also called IGFIIR/CI-MPR) [13–15]. In addition to its intracellular role in lysosome biogenesis and regulation of circulating IGF-II levels, the CI-MPR has been implicated in many other cellular functions due to the fact that it binds to Man-6-P-containing proteins that are not lysosomal hydrolases. These include transforming growth factor-β (TGF-β) precursor [16], the placental angiogenic hormone proliferin [17], the cytokine leukemia inhibitory factor [18], and the T-cell activation antigen CD26 [19], to name just a few. Functional roles of the cell surface CI-MPR in interactions with these non-lysosomal proteins include activation of TGF-β precursor and renin precursor [20] and clearance from the plasma in the case of leukemia inhibitory factor [21]. In addition to IGF-II, the CI-MPR has been shown to interact with a number of proteins that do not have phosphomannosyl residues. These include urokinase-type plasminogenactivator receptor (uPAR) [22], plasminogen [23], retinoic acid [24], and heparanase [25]. Residues essential for uPAR and plasminogen binding have been mapped to domain 1 [26] (Figure 2). However, the nature of their interactions is not clear.

In addition to the two MPRs, several proteins have been identified as containing mannose 6-phosphate receptor homology (MRH) domains [27], including erlectin (a luminal ER protein implicated in the regulation of glycoprotein trafficking) [28], the β-subunit of glucosidase II (also in ER), and GlcNAc-phosphotransferase γ-subunit (in Golgi), all of which are implicated in N-glycan recognition [29]. However, studies on their recognition of specific glycans are only beginning to emerge. Recently, human OS-9, which is involved in ER protein quality control, has been shown to interact with mannose-trimmed N-glycans [30]. However, studies will be needed to verify that the MRH domains of these proteins are structurally related to the MPRs.

This review will focus on recent structural studies of the CD-MPR and CI-MPR, with emphasis on how these receptors interact with phosphomannosyl residues. For other aspects of MPRs and related proteins, the reader is referred to articles by Ghosh et al.[31], Gary-Bobo, et al. [32], Dahms et al. [33], and Brown et al.[34].

Structures of the CD-MPR and CI-MPR

The MPRs are type 1 transmembrane glycoproteins (Figure 2). The CD-MPR forms a stable homodimer and the CI-MPR exists most likely as a dimer [35]. The bovine CD-MPR is composed of a 28-residue amino-terminal signal sequence, a 159-residue extracytosolic domain, a 25-residue transmembrane region, and a 67-residue carboxyl-terminal cytosolic domain. The six cysteine residues in the extracellular region of the CD-MPR form three disulfide linkages that play an important role in the folding of the molecule. The bovine CI-MPR contains a 44-residue signal sequence, a 2,269-residue extracytosolic region, a 23-residue transmembrane region, and a 163-residue cytosolic domain. The large extracytosolic region

consists of 15 contiguous domains, each of which, when compared to CD-MPR and to each other, has a similar size and significant amino acid sequence identity (14–38 %) including cysteine distribution, strongly suggesting that they have similar tertiary structures. Indeed, the crystal structures of the extracytosolic domain of the CD-MPR and domains 1, 2, 3, 11, 12, 13, and 14 of the CI-MPR all have the same fold (Figure 3) [36–39] (see below).

The structure of the extracytosolic domain of the CD-MPR in complex with Man-6-P and Mn^{2+} was first determined over 10 years ago [40,41] and has established the overall polypeptide fold of each domain in the P-type lectin family (Figure 3A). The molecule was found to be a dimer, consistent with the predominant oligomeric form of the CD-MPR found in membranes. The overall fold of the CD-MPR monomer is that of a flattened β-barrel consisting of two antiparallel β-sheets, one with four β-strands and the other with five strands, with strand 9 interjecting between strands 7 and 8. The dimer interface is formed by two five-stranded β-sheets. The structure of domains 1-3 (the N-terminal 432 residues) of the CI-MPR has been determined in complex with Man-6-P [36,42], confirming that the overall fold of each domain in the CI-MPR is the same as that of the CD-MPR (Figure 3). While the CD-MPR forms a dimer, the N-terminal three domains of the CI-MPR exist as a monomer. The three domains of the CI-MPR form a compact tri-lobed disk and have considerable contact with one another (contact areas between domains range from 8 to 22 %). These extensive interactions suggest that the observed three-domain arrangement forms a structural unit within the entire CI-MPR molecule[36], and that the interactions of domain 3 with the loop between domains 1 and 2 are required for maintaining the architecture of its sugar binding site.

Comparison of Man-6-P binding pockets in the CD-MPR and CI-MPR

The Man-6-P binding site in the CD-MPR is located at the C-terminal opening of the β-barrel (Figures 3 and 4). One side of the binding pocket is walled off by two loops (loop C, residues G98 – R108; loop D, E134 – C141) and the other side is exposed to the solvent, to provide room for the rest of the ligand molecule. The pocket is lined with residues Y45, D103, N104, H105, R135, E133, Y143, Q66, and R111, the latter four of which are found to be essential for Man-6-P binding by mutagenesis studies of CD-MPR [43–45] and domains 3, 5, and 9 of the CI-MPR [46,47] (Figure 4B). These four signature residues in both the CD-MPR (E133, Y143, Q66, and R111) and domain 3 of CI-MPR (E416, Y421, Q348, and R391) are absolutely conserved (Figure 4C) and interact with the Man-6-P ligand in the same manner, strongly suggesting that the sugar-binding pockets in domains 5 and 9 are also very similar to those of CD-MPR and domain 3 of the CI-MPR (Figure 4A). Indeed, the Man-6-P binding pocket located in domain 3 is essentially the same as that of the CD-MPR, with one exception: the absence of Mn^{2+} which accounts for the metal independence of the CI-MPR. In addition, solution structures of domain 5, which preferentially binds phosphodiesters, with and without a phosphodiester ligand have been determined (L Olson, abstract 216 in Glycobiology 18, 993, 2008). Preliminary results indicate that the overall fold of the isolated domain 5 is similar to that of the other CI-MPR domains and CD-MPR, and mutagenesis studies have demonstrated the essential role of these four signature residues in the lectin activity of domain 5 [47]. It is interesting to note that the IGF-II binding pocket is located in the homologous position in domain 11 as the Man-6-P binding pocket in domain 3 [37] (Figure 3D).

Inhibition studies using chemically synthesized oligomannosides or neoglycoproteins have shown that the presence of Man-6-P at a terminal position is the major determinant of receptor binding. Furthermore, linear glycans which contain a terminal Man-6-P linked α1,2 to the penultimate mannose were shown to be the most potent inhibitors [48,49]. Recent studies using a novel Phosphorylated Glycan Microarray demonstrate that the CD-MPR demonstrates a preference for glycans containing two phosphomonoesters, whereas the CI-MPR shows little

difference in affinity toward glycans containing one or two phosphomonoesters (X Song et al., unpublished).

The CD-MPR is dynamic – comparison of the ligand-bound and ligand-free CD-MPR

The MPRs travel between various cellular compartments during the transportation of their ligand, from TGN (where they bind the ligand) to late endosomes (where they release the ligand). Unlike the CI-MPR, the CD-MPR does not bind ligands at the cell surface. It has been shown that this loading/unloading is facilitated by changes in the pH of each compartment [50]. In order to understand the mechanism of the pH dependence of the ligand binding/release, crystal structures of the CD-MPR have been obtained at various pHs and in the presence and absence of bound Man-6-P [51,52]. Unexpectedly, regardless of pH, the receptor molecule adopts two conformations: an “open” conformation found in all structures with bound Man-6-P and a “closed” conformation observed in all structures without bound ligand (Figure 5). There are three major structural differences between these two states. First, in the open state (i.e., the bound state), the two ligand-binding sites of the dimeric molecule are ~35Å apart, whereas in the closed (unliganded) state, they are ~26 Å apart. Second, the architecture of the ligand binding pocket in the closed form differs significantly from the open form (Figure 5B). When the ligand is bound, loop D (residues E134-C141) forms one wall of the binding pocket. When the ligand is absent, loop D folds into the binding pocket, occupying the same space where the sugar molecule binds and thereby keeps the position of the sugar-binding residues unchanged. Lastly, this loop D movement disrupts electrostatic interactions between the two subunits, releasing inter-subunit salt bridges, causing the two subunits to slide and twist at their interface which results in the “closed” form (Figures 5B and 5C). Thus, the movements involved in the bound-to-unbound transition of the CD-MPR are reminiscent of those in the oxy-to-deoxy transition of hemoglobin. However, whether the CD-MPR has ligand-binding cooperativity has not been studied. The overall movement of the bound-to-unbound transition can be described as a “scissoring and twisting” motion between the two subunits at the dimer interface. The implication of the existence of these two states is that the CD-MPR must be able to readily transition between these two conformations as it travels to the different cellular compartments, with the unique environment of each compartment (*e.g.*, Golgi, cell surface, endosome) impacting the equilibrium between the two states.

Mechanisms of ligand binding and release by the CD-MPR

From the above structural studies, plausible mechanisms for the dissociation of lysosomal enzymes in the acidic endosome (pH, < 6.0) and at the cell surface (pH, 7.4) have been proposed [52]. The structure of the bound form of CD-MPR has three inter-subunit ion pairs that “tie” loop D of one subunit to the other subunit (K18 and E19 of one subunit to E134 and K138 of the other subunit, respectively) (Figure 5D). In the acidic environment of the endosome, the disruption of inter-subunit electrostatic interactions may trigger the ligand release by protonation of the carboxyl groups of the glutamates and aspartate (Figures 5D and 5E). In addition, E133, one of the essential residues for binding of Man-6-P, might also be protonated in the endosome and weaken its binding to Man-6-P, promoting release of the bound ligand (Figure 5D). On the other hand, at the cell surface, deprotonation of H105 and the phosphate moiety of Man-6-P (both of which interact with each other in the bound conformation) are most likely responsible for the release of the ligand (Figures 4 and 5C). To probe directly the role of the electrostatic interactions between the two subunits, specifically the salt-bridge between E19 and K137, a double mutant (E19Q/K137M) has been analyzed by surface plasmon resonance. The mutant CD-MPR lacking the inter-subunit salt bridge binds lysosomal enzymes with ~100-fold lower affinity, clearly demonstrating the critical role of the ionic interactions between the two subunits in stabilizing the bound conformer of the CD-MPR.

Conclusions

Although we have accumulated considerable knowledge on the MPRs regarding the structural basis for their phosphomannosyl recognition and the mechanisms of the cargo loading and unloading for the CD-MPR, there still remain many outstanding questions. It is interesting to note that all ligand binding sites of CI-MPR are located at odd-numbered domains, i.e., domains 3, 5, and 9 for Man-6-P binding and domain 11 for IGF-II, as well as domain 1 which is found to interact with plasminogen and uPAR. What are the roles of the remaining domains? Do even-numbered domains function only as spacers and/or support for the functional domains? What are the mechanisms of ligand binding and release for the CI-MPR, which must be very different from those of CD-MPR? Answers to these questions must await further biochemical and structural studies of the CI-MPR including fragments containing overlapping ranges of domains. It is also intriguing that the CI-MPR complements the two enzymes that make the phosphomannosyl tag by being able to bind to the products of both the first and the second enzymes of the two-step process. Is this purely for redundancy? Furthermore, the mechanisms that govern the MPRs' trafficking to different subcellular compartments are not clearly known. MPRs are present in TGN, endosomes, and plasma membrane, but absent in lysosomes. They cycle constitutively between these compartments and this trafficking is directed by sorting signals that reside in the cytosolic tails of the receptors. Currently, only the structures of peptides (12 or 13 residues) that contain the di-leucine motif in the cytosolic tails of CD-MPR and CI-MPR, in complex with the VHS domain of human GGA (Golgi-localized, -γ-ear-containing, ADP-ribosylating-factor-binding) proteins are known [53,54]. Thus, in order to gain a clearer picture of the factors that regulate the interaction of the MPRs' cytoplasmic region with numerous cytosolic adaptor proteins, further structural analyses are needed.

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Glossary

MPR
: mannose 6-phosphate receptor

CD-MPR
: cation-dependent MPR

CI-MPR
: cation-independent MPR

Man-6-P
: mannose 6-phosphate

TGN
: trans Golgi network

MRH
: MPR homology

uPAR
: urokinase-type plasminogen activator receptor

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• Of special interest

•• Of outstanding interest

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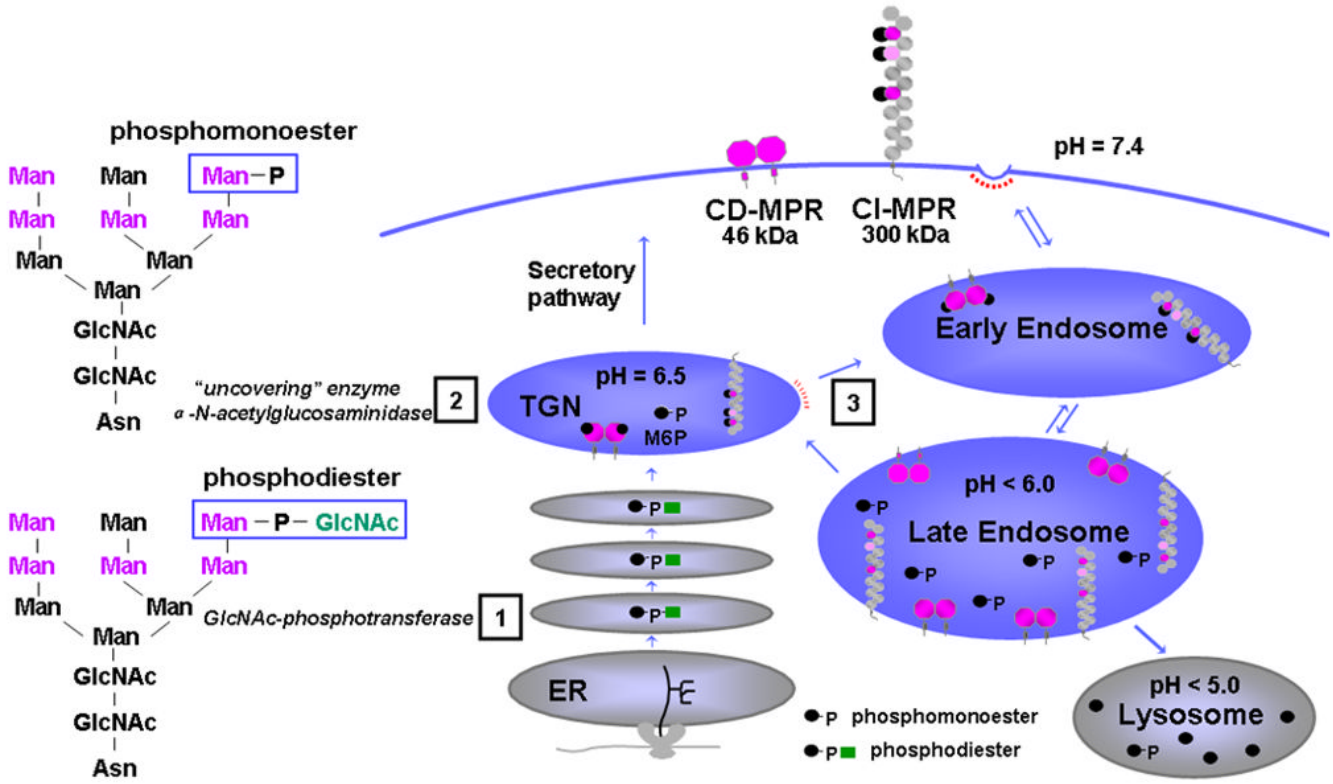


Figure 1.

Lysosomal enzyme trafficking. Movements of lysosomal enzymes and MPRs between the various intracellular compartments and the cell surface are shown. Phosphorylation of mannose residues on *N*-linked oligosaccharides occurs in two steps (see text). The five potential sites of phosphorylation are indicated in pink letters. Lysosomal enzymes that acquire the Man-6-P tag in early Golgi compartments bind specifically to MPRs in the Golgi. The resulting receptor-lysosomal enzyme complex is transported from the trans Golgi network (TGN) to an early endosomal compartment (step 3) and to an acidified late endosomal compartment where the low pH of the compartment causes dissociation of the complex. Lysosomal enzymes that are not phosphorylated (•), contain a phosphomonoester (•-P), or contain a diester with N-acetyl glucosamine are shown (•-P-■). The dimeric CD-MPR is depicted as two pink balls and the CI-MPR is shown as 15 repeating balls. The three Man-6-P binding domains of the CI-MPR are depicted as pink balls.

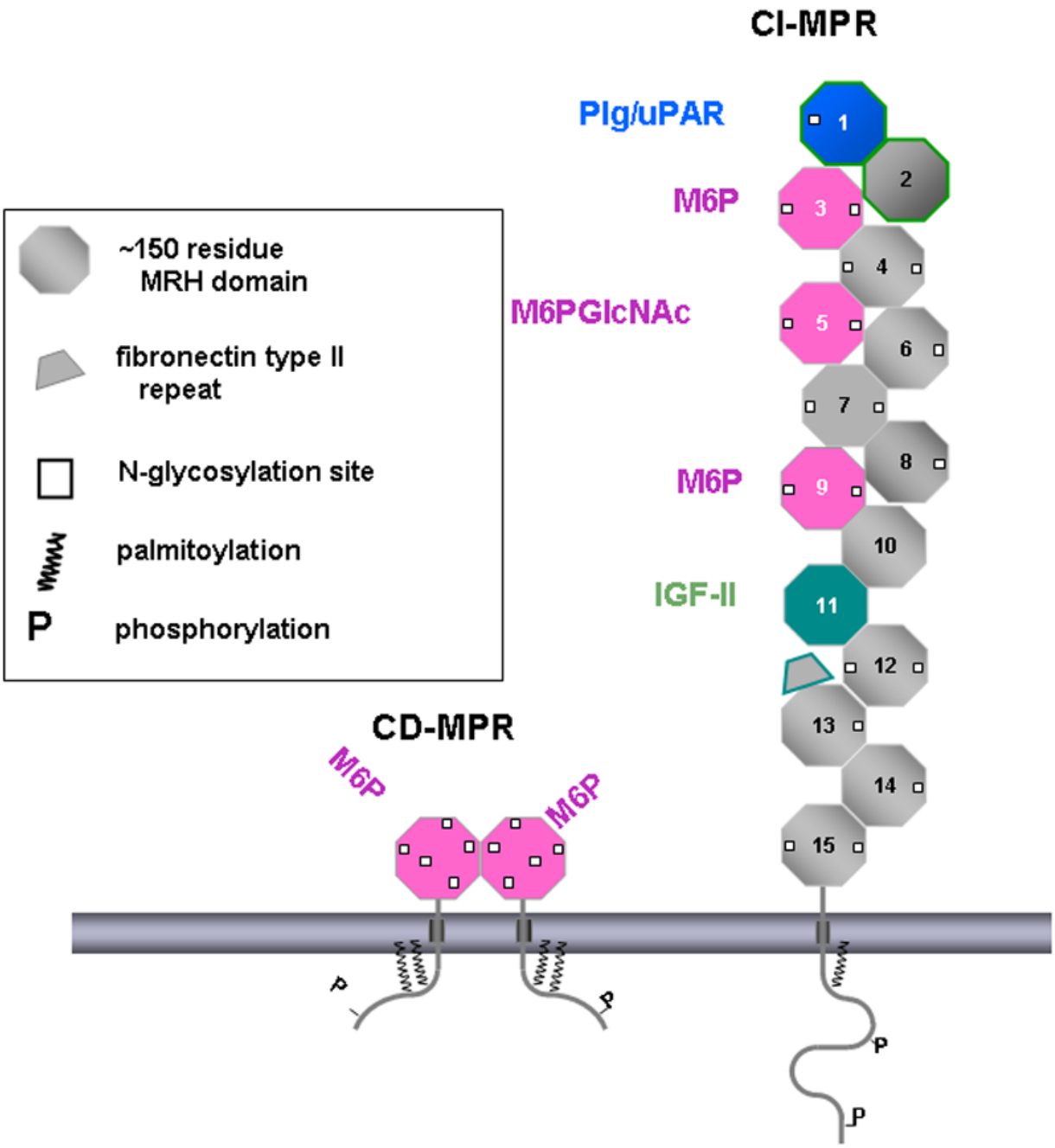


Figure 2.

Schematic representation of the CD-MPR (left) and CI-MPR (right). The MPRs are transmembrane glycoproteins. Various post-translational modifications are indicated, including palmitoylation and phosphorylation. The CD-MPR is shown as a dimer with each subunit having one Man-6-P binding site. The 15 repeating domains of the CI-MPR are numbered sequentially from the N-terminus to C-terminus. Domains 3, 5, and 9 bind Man-6-P with domain 5 preferentially binding to phosphodiesters. Domain 1 is known to bind to urokinase plasminogen activator receptor (uPAR) and plasminogen (Plg). Domain 11 binds IGF-II and Domain 13 has a fibronectin II insert.

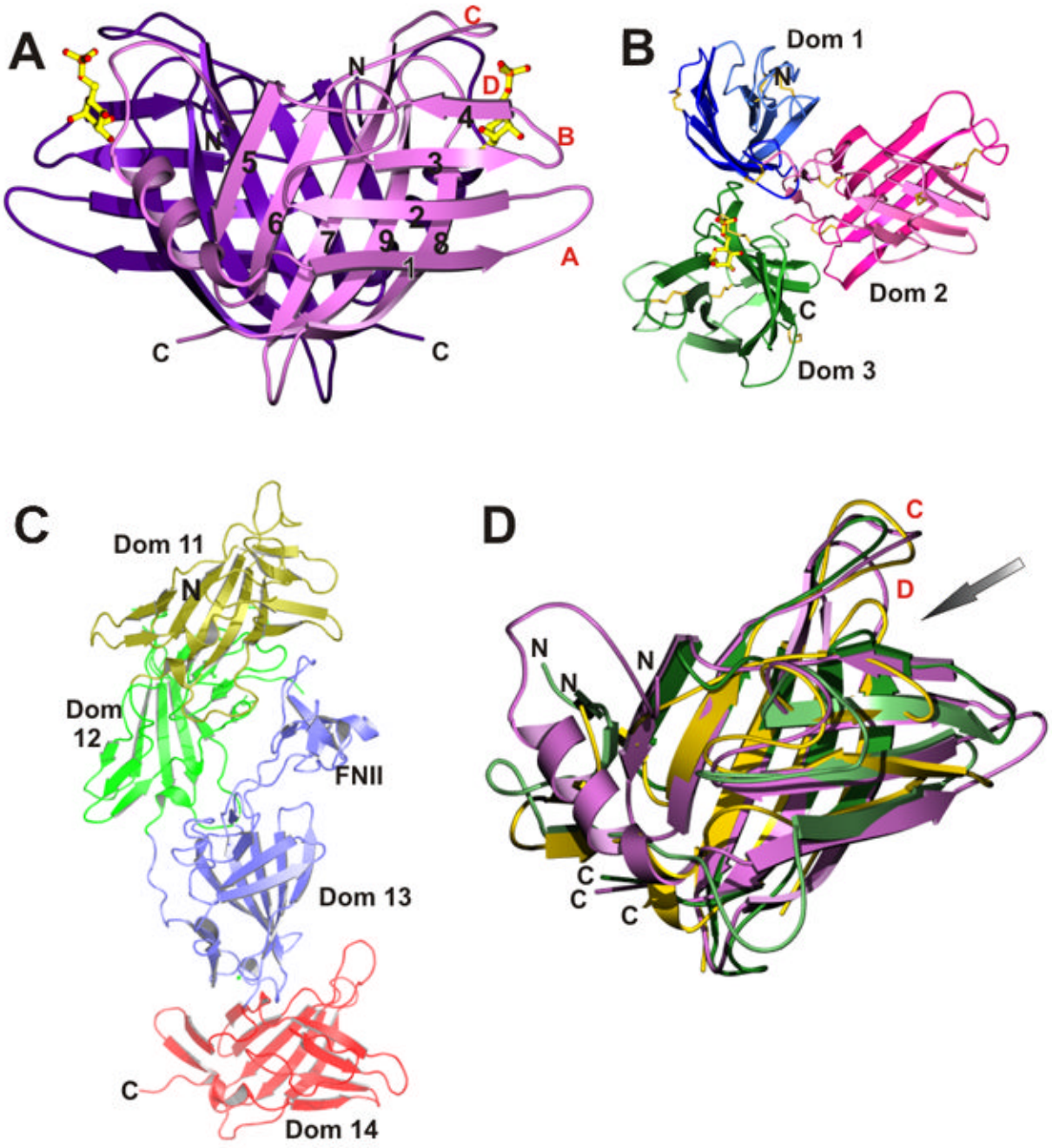
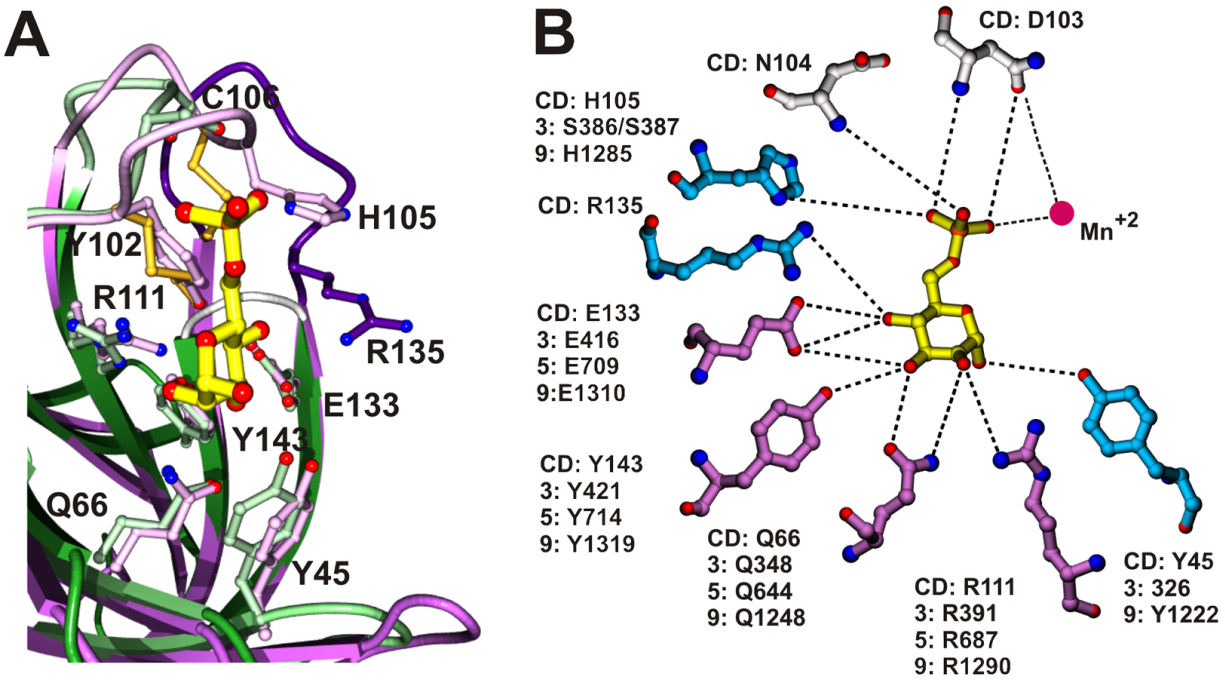


Figure 3.

A collage of structures of the CD-MPR and CI-MPR. A) Dimer of the CD-MPR. β-Strands are numbered from N- to C- terminus and loops between strands are labeled in alphabetic order. B) Structure of domains 1–3 of CI-MPR, with bound Man-6-P in domain 3. C) Structure of domains 11–14 of the CI-MPR. N- and C-termini are indicated. FNII denotes the fibronectin II insert in domain 13. D) Superposition of the structures of the CD-MPR (monomer, purple) and domains of 3 (green) and 11 (gold) of the CI-MPR, demonstrating that they all have a similar polypeptide fold. For clarity, the structures of domains 1, 2, 12, 13, and 14, whose structures have been determined to have the same fold, are not included in the overlay. The ligand binding site is indicated with an arrow.



C

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                                  -2β          -1β                  β1          β2
CI-MPR-3    282----RDYLESRSCSLSSAQHDVAVDLQPLSRVEASDSLFYTSEADEYTYYLSICGGSQAP
CI-MPR-5    584------RTEGDNCTVFDSQAGFSFDLTPLTKKDAY-----KVETDKYEFHINVCGPVSVG
CI-MPR-9    1184----VVRAEGDYCEVRDPRHGNLYNLIPLGLNDT------VVRAGEYTYYFRVCGELTSG
CD-MPR       3TEEKTCDLVGEKGKESEKELALLKRLTPLFNKSFES--TVGQSPDMYSYVFRVCREAGN-
CI-MPR-11   1511------SNVHDDCQVTNPATGHLFDLSSLSGRAGFT----AAYSEKGLVYLSVCGDNEN-

                                  β3          β4                  β5          β6
CI-MPR-3    338ICNKKDAAVCQVKKADSTQVK----VAGRPQNLTLRYS-DGDLTLIYFGGEECS---SGF
CI-MPR-5    635ACPP-DSGACQVSRSDRKSWN------LGRSNAKLSYY-DGMIQLTYRDGTPYNN-EKRT
CI-MPR-9    1234VCPT-SDKSKVISSCQEKRGPQGFQKVAGLFNQKLTYE-NGVLKMNYTGGDTCH---KVY
CD-MPR       58--HSSGAGLVQINKSNGKETV------VGRFNETQIFNGSNWIMLIYKGGDEYDNHCGRE
CI-MPR-11   1559-CANGVGACFGQTRIS----------VGKASKRLTYV-DQVLQLVYEGGSPCPS-KTGL

                                  β7          β8                  β9
CI-MPR-3    390QRMSVINFECNQTAGNNGRGAPVF--GEVD-----CTYFFTWDTKYACV432
CI-MPR-5    689PRATLITFLCDRDAG---VGFPEY--EEDN-----STYNFRWYTSYACP725
CI-MPR-9    1289QRSTTIFFYCDRST----QAPVFLQ--ETS----DCSYLFEWRTQYACP1328
CD-MPR      110QRRAVVMISCNRHTLADNFNPVS----EERGKVQDCFYLFEMDSSLACS154
CI-MPR-11   1613SYKSVISFVCRPEVGPTNRPMLISL--DKR----TCTLFFSWHTPLACE1647
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Figure 4.

Conservation of the Man-6-P binding site and essential residues for carbohydrate binding. A) Superposition of the Man-6-P binding sites of the CD-MPR (purple) and domain 3 of the CI-MPR (green). The architecture of both binding pockets is essentially the same with the exception of loop D (dark blue, CD-MPR; grey, domain 3), which is shorter in domain 3. B) A schematic drawing showing interactions between Man-6-P and residues in the CD-MPR and their homologous residues in domains 3, 5, and 9 of the CI-MPR. Dotted lines indicate potential hydrogen bonds. Mutational studies have shown that the four residues shown in purple are essential for Man-6-P binding and that mutation of the residues shown in blue partially decreased Man-6-P binding affinity. The two residues in grey have not been tested. C) Sequence

alignment of domains 3, 5, 9 and 11 of the CI-MPR and the extracytosolic domain of the CD-MPR. The conserved cysteines are highlighted in yellow and the four residues that are essential for Man-6-P binding are highlighted in red. Residues that are within hydrogen bonding distance of Man-6-P in the crystal structures of CD-MPR and domains 1–3 of the CI-MPR, but found to be not essential for Man-6-P binding are boxed in red. Secondary structural elements are indicated with arrows and the single α-helix found in the CD-MPR is indicated with a green cylinder. Residues involved in IGF-II binding in domain 11 of the CI-MPR are boxed in blue.

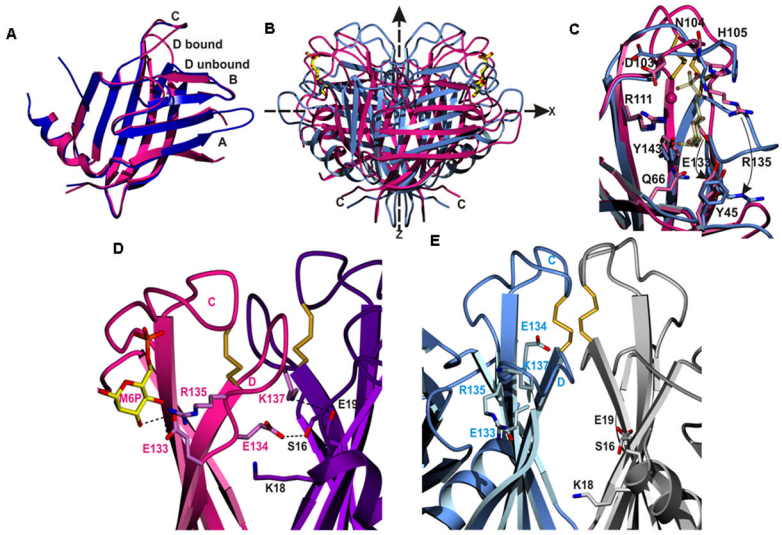

Figure 5.

Comparison of the ligand-bound and ligand-free structures of CD-MPR. A) Superposition of the monomer structures of the bound (red) and unbound (blue) structures. Note the difference in the conformations of loop D. B) The dimer structures, with the same color scheme as in (A). The molecule is scissoring along its molecular two-fold axis (z-axis) and twisting along the x-axis. The ligand, Man-6-P, is shown with ball-and-sticks. C) Superposition of the ligand binding sites of the bound (red) and unbound (blue) structures. Loop D in the bound structure forms the side of the binding pocket, while in the unbound structure loop D folds down and occupies the Man-6-P binding site. Mn^{+2} ion in the bound state is denoted as a red ball. The movements of E133 and R135 are indicated (curved arrows). Inter-subunit interfaces are shown, including the N-terminus and loop D of the bound (panel D) and unbound (panel E) structures. Electrostatic interactions found between the two subunits in the bound structure are disrupted in the unbound structure, resulting in a weaker dimer interface.