MANNOSE 6-PHOSPHATE RECEPTORS: NEW TWISTS IN THE TALE

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The two mannose 6-phosphate (M6P) receptors were identified because of their ability to bind M6P-containing soluble acid hydrolases in the Golgi and transport them to the endosomal–lysosomal system. During the past decade, we have started to understand the structural features of these receptors that allow them to do this job, and how the receptors themselves are sorted as they pass through various membrane-bound compartments. But trafficking of acid hydrolases is only part of the story. Evidence is emerging that one of the receptors can regulate cell growth and motility, and that it functions as a tumour suppressor.

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The two mannose 6-phosphate (M6P) receptors (MPRs) — the ~46-kDa cation-dependent MPR (CD-MPR) and the ~300-kDa cation-independent MPR/insulin-like growth factor-II (IGF-II) receptor (CI-MPR) — are the sole members of the family of p-type lectins[1]. The CI-MPR is a multifunctional receptor that carries out several tasks that are essential for normal cellular function. One such task, which is shared with the CD-MPR, is the delivery of newly synthesized acid hydrolases from the *trans*-Golgi network (TGN) to endosomes for their subsequent transfer to lysosomes. This process involves binding of the hydrolases, through their M6P-recognition moieties, to the receptors, packaging of the ligand–receptor complexes into carriers that transport their cargo to target endosomes and recycling of the receptors back to the TGN.

In addition to this shared function, the CI-MPR has been implicated in several other physiological processes. It binds IGF-II at the cell surface and internalizes this growth factor for degradation in lysosomes. This prevents the accumulation of excessive levels of IGF-II, which are detrimental, especially during embryonic development. It facilitates the activation of the latent precursor of transforming growth factor-β1 (TGF-β1) and it mediates the uptake of granzyme B, which is a serine protease involved in cytotoxic-T-cell-induced apoptosis. There is also evidence indicating that the CI-MPR acts as a tumour suppressor, probably through these growth-inhibitory functions. Moreover, insights have been gained into the molecular mechanisms that govern carbohydrate and IGF-II recognition by the MPRs, and the cellular components that mediate the transport of the receptors through numerous intracellular compartments. This review focuses on these recent findings. Other reviews summarize earlier studies of the MPRs[2–4] and the crystal structure of the CD-MPR[1].

The structures of the MPRs

The two MPRs are type-1 integral membrane glycoproteins (FIG. 1). The extracytoplasmic region of the CI-MPR has a repetitive structure that consists of 15 contiguous repeats of approximately 147 amino acids each. The repeating segments share sequence identity (14–38%) and cysteine distribution, which gives rise to the possibility that they have similar disulphide-bonding and tertiary structures[5]. The 159-residue extracytoplasmic domain of the CD-MPR is similar to the repeating units of the CI-MPR.

The CI-MPR extracytoplasmic domain contains two distinct M6P-binding sites (repeating segments 3 and 9) and a single IGF-II-binding site (segment 11)[6–9], whereas the CD-MPR contains a single M6P-binding site and does not bind IGF-II. The cytoplasmic tails of both receptors contain numerous sorting signals, some

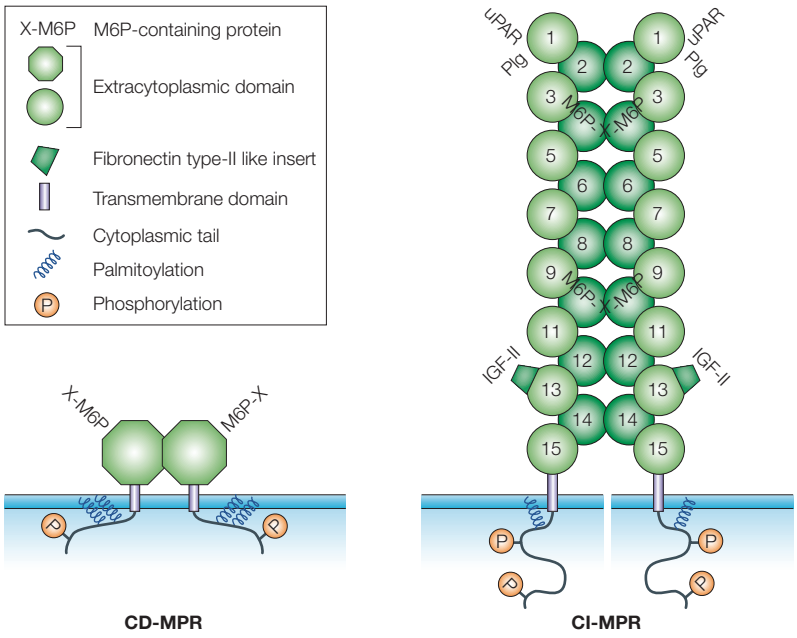

Figure 1 | **The MPRs are type-I transmembrane glycoproteins.** The cation-dependent mannose 6-phosphate (M6P) receptor (CD-MPR) is present predominantly as a stable homodimer in membranes and has a single M6P-binding site per polypeptide. The cation-independent (CI)-MPR seems to be a dimer in the membrane, although it tends to act as a monomer in detergent solutions. Various post-translational modifications of the MPRs occur, including palmitoylation and phosphorylation. uPAR, urokinase (plasminogen activator) receptor; IGF-II, insulin-like growth factor; Plg, plasminogen.

of which are modified by phosphorylation[10,11] or palmitoylation[12]. The CD-MPR is present primarily as a non-covalent homodimer in the membrane[1]. The CI-MPR also seems to be a dimer in the membrane, although it behaves as a monomer in detergent solutions under most circumstances[13–15]. Receptor dimerization allows for high-affinity binding of ligands that are multivalent for M6P residues[14–15].

Important insights into the function of the MPRs have come from X-ray crystallographic studies of the three-dimensional structure of the extracytoplasmic region of the CD-MPR, both in the unliganded state and complexed to either M6P or pentamannosyl phosphate[16–18]. In both liganded and unliganded forms, the molecule crystallized as a homodimer with approximately 20% of the entire surface area of each monomer having contact with another through predominantly hydrophobic interactions (FIG. 2a). Each monomer contains a single α-helix near its amino terminus followed by nine primarily anti-parallel β-strands that form two β-sheets, which are positioned orthogonally to each other. Extensive hydrophobic interactions are formed between the two β-sheets, which results in each monomer forming a flattened β-barrel structure.

The six cysteine residues form three intramolecular disulphide bonds that are essential for the ligand-binding conformation of the receptor to be generated. The structures of the liganded molecules show that the carbohydrate-recognition domain (CRD) of the CD-MPR lies relatively deep inside the protein, so that the terminal M6P residue and the penultimate sugar ring of bound pentamannosyl phosphate are mostly buried in the receptor[17]. This deep binding pocket facilitates the formation of numerous interactions between the CD-MPR and its carbohydrate ligands. The structure of the ligand-free receptor differs considerably from the liganded receptor molecule (FIG. 2a,b). This indicates that the 'free-to-bound' transition requires the receptor monomer to undergo significant scissoring and twisting movements, such that the 'closed' ligand-free conformation is 'opened' up to allow ligand binding[18].

A structure-based sequence alignment between the CD-MPR and domains 3 and 9 of the CI-MPR provides evidence that both receptors use a similar carbohydrate-recognition mechanism[16]. This was confirmed by site-directed mutagenesis studies, which showed that the two binding sites of the CI-MPR use the same essential amino acids for ligand binding[6] (FIG. 2c). However, the M6P-binding domains of the CI-MPR lack a residue that is analogous to aspartic acid 103, which coordinates divalent cations, explaining why only the CD-MPR shows enhanced ligand binding in the presence of divalent cations.

The overall structure of the IGF-II binding domain 11 of the CI-MPR is similar to that of the CD-MPR[19] (FIG. 3a,b). However, the molecule contains a surface hydrophobic patch that equates spatially to the hydrophilic M6P-binding pocket on the CD-MPR, explaining the lack of carbohydrate binding by CI-MPR domain 11 (FIG. 3c,d). This hydrophobic patch is probably involved in IGF-II binding as it contains isoleucine 1572, which is required for this interaction[9]. As the residues on IGF-II that are essential for binding to the CI-MPR form a hydrophobic patch on the surface, it is probable that the interaction of IGF-II with the CI-MPR is predominantly hydrophobic. This is similar to the interaction of the homologous IGF-I with IGF-binding protein 5 (REF. 20).

The short linker length between the CI-MPR repeating segments (5–12 residues) places considerable constraints on possible arrangements of the domains in the intact receptor, and Brown *et al.*[19] have proposed a model in which even-numbered domains face one direction and odd-numbered domains face the opposite direction (FIG. 1). In this model, the putative IGF-II binding face of domain 11 is adjacent to the region of domain 13 that contains a fibronectin type II-like insert, which contributes to the enhancement of IGF-II binding by domain 13 (REFS 21,22). As all of the known functional domains of the CI-MPR have odd numbers, it is possible that one side of the molecule is involved in ligand interactions, whereas the opposite surface has another role, such as mediating dimerization. This would be similar to what has been found with the CD-MPR (FIG. 2).

MPR trafficking — the itinerary

MPRs are found in the TGN, early (sorting) endosomes, recycling endosomes, late endosomes and the plasma membrane, but they are conspicuously absent from lysosomes (FIG. 4). The receptors cycle

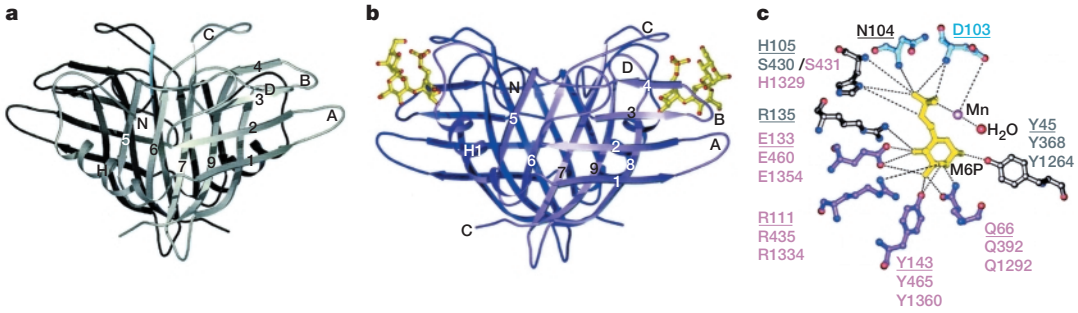

Figure 2 | **The crystal structure of the extracytoplasmic region of the bovine CD-MPR. a** | The non-ligand-bound form of the cation-dependent mannose 6-phosphate (M6P) receptor (CD-MPR) shows several significant conformational changes as compared to **b** | the ligand-bound form. In the ligand-free form, loop D — which encompasses residues E134–C141 — bends into the unoccupied M6P-binding cleft. This reorientation of loop D results in the formation of an intricate network of hydrogen bonds that seems to maintain the integrity of the binding site and keep the side chains of three of the four residues that are essential for carbohydrate recognition in position to bind the ligand. The phosphate group and the three terminal mannose rings of the pentamannosyl phosphate are depicted in yellow 'ball-and-stick model' form. **c** | A schematic view of the potential hydrogen bond and ionic interactions between the binding-pocket residues of the CD-MPR and the phosphate group and the terminal mannose ring of the pentamannosyl phosphate. Directly beneath the CD-MPR residue designations (underlined) are listed the corresponding carbohydrate-binding-site residues of domain 3 and domain 9 of the extracytoplasmic region of the cation-independent M6P/insulin-like growth factor II (IGF-II) receptor (CI-MPR), as predicted by the CD-MPR structure-based sequence alignment. Those residues that are essential for high-affinity M6P binding by the MPRs are shaded purple. Parts **a** and **b** are modified with permission from REF. 18 © the American Society for Biochemistry and Molecular Biology (2002). Part **c** is modified with permission from REF. 1 © Elsevier Science (2002) and REF. 17 © the American Society for Biochemistry and Molecular Biology (1999).

ADAPTORS
Heterotetrameric protein complexes that connect molecules on membranes and structural coat proteins, for example clathrin. AP1 mediates cargo transport between the *trans*-Golgi network (TGN) and endosomes through the generation of clathrin-coated carriers.

CLATHRIN-COATED BUDS AND VESICLES
These are membrane evaginations that have assembled clathrin on their surface. The forming vesicles bud off and function as carriers for sorted proteins.

GGA
A multidomain, cytosolic protein with an amino-terminal VHS domain that binds cargo, followed by a coiled-coil GAT domain that mediates membrane recruitment through ARF; a variable hinge segment that contains clathrin- and AP1-binding regions; and a carboxy-terminal ear domain, which recruits accessory proteins and has homology to the γ-appendage. These molecules have recently been implicated in the sorting of MPRs at the TGN.

VHS DOMAIN
An approximately 150-residue domain whose name is derived from its presence in VPS-27, Hrs and STAM. It is found at the amino termini of proteins that are associated with endocytosis and/or vesicular trafficking.

CK-2 SITE
(casein kinase 2).
A phosphorylation site that is characterized by the presence of phosphoacceptor residues (S/T) that are flanked by clusters of negatively charged amino acids, the residue at position n+3 being one of the following: an aspartate, glutamate, phosphoserine or phosphothreonine. The minimum concensus sequence is S/T XX D/E/pS/pT.

constitutively between these organelles and avoid being delivered to lysosomes in which they would be degraded. This trafficking is directed by several sorting signals that are present in the cytoplasmic tails of the receptors (FIG. 5).

Packaging at the TGN. The TGN is the site where newly synthesized acid hydrolases that are destined for the endosomal–lysosomal system are sorted away from secretory proteins. The synthesis of the M6P-recognition signal on the acid hydrolases is completed in the late-Golgi compartments[23]. This allows acid hydrolases to bind to MPRs — the first step in the sorting process. Early immunolocalization studies[24] detected the MPRs in ADAPTOR complex AP1-positive CLATHRIN-COATED BUDS AND VESICLES in the TGN, and pulse–chase labelling experiments showed that the acid-hydrolase–MPR complexes exit the TGN in clathrin-coated vesicles (CCVs)[25,26]. Analysis of receptor-deficient cell lines that were expressing mutant MPRs showed that efficient lysosomal enzyme sorting by this intracellular pathway is dependent on an acidic-cluster–dileucine (AC–LL) motif near the carboxyl terminus of the cytoplasmic tails of the receptors[27–30]. This led to the assumption that the MPRs bind to AP1 through the AC–LL motif. It was surprising, therefore, when it was reported[31,32] that mutation of the dileucine pair of the CD-MPR did not impair binding to AP1.

GGAs and AP1 capture cargo. Discovery of the Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding proteins (GGAS) — a family of multidomain proteins — clarified the situation[33–37]. The three mammalian GGAs — GGA1, GGA2 and GGA3 — are recruited from the cytoplasm onto the TGN by ADP-ribosylation factor–GTP (ARF•GTP), and bind to the crucial AC–LL motif through their VHS DOMAINS[38–40]. Sorting-defective MPRs with mutations in this motif fail to bind to the GGAs, which indicates that this interaction is important in the targeting pathway[39].

Although the AC–LL signals of both CD-MPR and CI-MPR contain the minimal motif required for VHS binding (D/EXXLL[41–43], where X is any amino acid; FIG. 6), the CD-MPR binds with less avidity to the VHS domains of GGAs than does the CI-MPR[38,40]. This is due to several non-conservative amino-acid differences in the AC–LL motifs and the flanking residues that are found in the cytoplasmic tail[44]. The hinge region of the GGAs binds clathrin[39,45], which raises the possibility that the GGAs might nucleate CCVs.

In contrast to the single AC–LL binding motif for the GGAs, the cytosolic tails of both MPRs have many binding sites for AP1 (FIG. 5). The CD-MPR contains two independent sequences (amino acids 28–42 and 49–67) that bind AP1 with similar low-nanomolar affinities[32]. The dileucine pair at positions 64–65 is not required for AP1 binding, in contrast to the requirement for these residues in GGA binding. There are at least four binding sites for AP1 on the CI-MPR tail[46,47] (P.G. and S.K., unpublished data): the 26YSKV29 motif, the internal 'dileucine' motif (39ETEWLM44), and the two casein kinase 2 (CK-2) SITES (84DSEDE88 and 154DDSDED160). The avidity of AP1 for the CK-2 sites is enhanced significantly on phosphorylation[46,47] (P.G. and S.K., unpublished data). Although there is strong evidence that YXXϕ motifs (where ϕ is a bulky hydrophobic residue) bind to the μ-subunits of AP1 and AP2 (REFS 48,49), there is a debate as to whether dileucine motifs bind to the μ- or the β-subunits[50,51]. It is also uncertain where the acidic clusters bind, although the crystal structure of μ2

shows that there is a striking positive surface electrostatic potential that might allow interactions with the acidic clusters[49].

Cooperate to operate

Two proposals have been put forward to explain why GGAs are required for MPR sorting, even though these receptors can bind directly to AP1. One is that GGAs and AP1 function in parallel by nucleating separate vesicular carriers, which are perhaps destined for distinct endosomal compartments as indicated by genetic evidence in yeast[52–54]. Alternatively, the GGAs could facilitate MPR entry into AP1 CCVs. A possible clue to this function lies in the architecture of the TGN, as shown by high-resolution ELECTRON TOMOGRAPHY[55,56].

The Golgi is composed of stacked cisternae. The last two or three stacks, with their tubulo-vesicular extensions, make up the *trans*-Golgi/TGN (FIG. 7). Tubules that originate from the *trans*-most stack (C7) seem to produce exclusively clathrin-coated buds, whereas those from stacks C5 and C6 produce non-clathrin-coated buds. As the MPRs with their bound acid hydrolases exit the TGN in clathrin-coated carriers[25,26], there must be a mechanism for preventing these complexes from entering transport carriers that originate from C5 and C6, which are presumed to be destined for the plasma membrane.

The GGAs seem to be well positioned to mediate this function. Quantifiable cryo-immunogold studies have shown that 50% of the GGA is present on non-coated membranes, which is consistent with its encountering the MPRs on the smooth membranes of the C5 and C6 cisternae[57]. The GGAs could retain the MPRs in the Golgi and allow them to reach the *trans*-most cisternae, where they colocalize with AP1 in clathrin-coated buds[57]. Within these clathrin-coated buds, GGAs seem to interact directly with AP1 through the binding of their hinge domains to the AP1 γ-appendage[57]. The ability of the GGAs to bind clathrin could strengthen this association. AP1, in turn, has an associated CK-2, which phosphorylates serine residues that are located just upstream of internal AC–LL motifs in the hinge domains of GGA1 and GGA3 (REFS 57,58). On phosphorylation, these AC–LL sequences can bind to the ligand-binding site in the VHS domain. This could induce the release of the bound MPRs and result in their directed transfer to AP1. The GGAs could then return to the cytoplasm, and this would account for the failure to detect these molecules in isolated CCVs[34]. This colocalization would not occur if the GGAs and AP1 nucleated only their own coated vesicles. However, the possibility that GGAs and AP1 also form separate transport vesicles cannot be excluded.

In this model, mutant MPRs that fail to bind GGAs would escape from the Golgi through the tubules of C5/C6 and never encounter AP1 in the terminal cisternae. By contrast, mutant GGAs that bind MPRs but do not interact with AP1 would trap the receptors in the TGN. In keeping with this hypothesis, it was observed[57] that MPRs that are defective in binding to GGAs are poorly incorporated into AP1-containing clathrin-coated buds. Puertollano *et al.*[38] showed that truncated GGA1, which lacks the hinge region and is therefore defective in its ability to bind to AP1, traps the MPRs in the TGN. These observations support the idea that the GGA–AP1 interaction, with the proper transfer of the receptor from one to the other, is essential for normal MPR trafficking.

Live video-microscopy has shown the dynamic nature of the TGN-derived tubules and raised questions about the fate of MPRs within these structures[38,59,60]. As discussed, these receptors could leave the tubules through CCVs[25,26,59], the rate of formation of which might be regulated by MPR concentration[61]. In addition, the TGN-derived tubules and their tubulo-vesicular fragments could themselves be carriers of sorted receptor–hydrolase complexes[60]. In this case, relatively larger-sized MPR-containing tubulo-vesicular carriers could fuse directly with endosomes to deliver their cargo. The AP1 clathrin-coated buds could function as traps for concentrated MPRs, preventing them from retreating back into the Golgi. It is possible that both methods of receptor routing occur simultaneously, and that one might be more dominant than the other depending on the cell type.

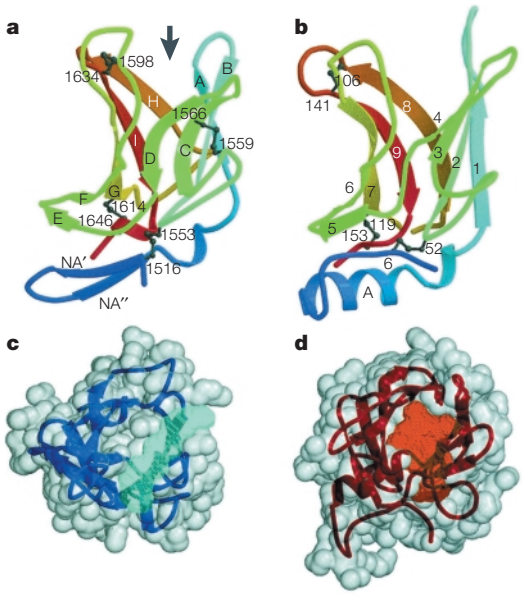

Figure 3 | **Comparison of the CI-MPR domain 11 and CD-MPR structures. a** | A ribbon diagram of the cation-independent mannose 6-phosphate (M6P)/insulin-like growth factor II (IGF-II) receptor (CI-MPR) domain 11 (Dom11; protein databank (PDB) accession code 1GP0, 1GP3), which shows that the flattened β-barrel structure is capped off by a β-hairpin. The arrow indicates the putative IGF-II-binding region. Ribbons are coloured from blue at the amino terminus to red at the carboxyl terminus, and disulphide linkages are represented in grey 'ball-and-stick' form[19]. **b** | A ribbon diagram of the cation-dependent (CD)-MPR (PDB accession code 1C39) coloured using the same scheme as in **a**. **c** | The proposed IGF-II-binding cavity (pale green) of CI-MPR Dom11 (grey surface and dark blue secondary structure). **d** | The M6P-binding cavity (orange) of CD-MPR (grey surface and red secondary structure). Reproduced with permission from REF. 19 © Oxford University Press (2002).

ELECTRON TOMOGRAPHY
A general method for the three-dimensional reconstruction of single, transparent objects from a series of projection images (that is, from a tilt series) that are recorded with a transmission electron microscope.

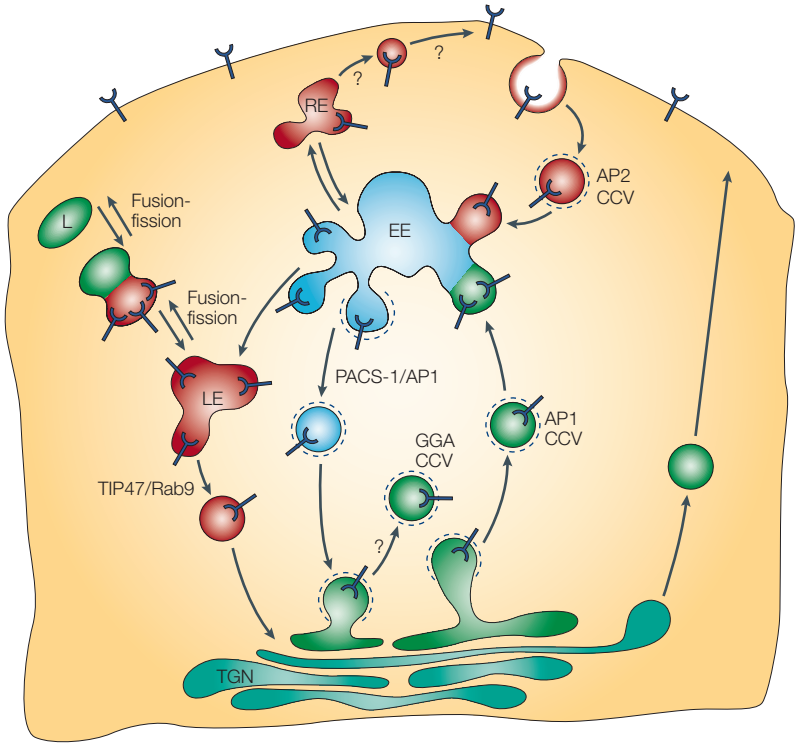

Figure 4 | **A schematic representation of the subcellular localization and trafficking itinerary of the MPRs.** Mannose 6-phosphate receptors (MPRs) bind to their cargo (acid hydrolases) in the *trans*-Golgi network (TGN), and are packaged into transport carriers that deliver the receptor with its bound ligand to early endosomes (EE). The low pH within the endosomes facilitates the dissociation of the acid hydrolases from the MPRs. Dynamic fusion/fission between the late endosomal and lysosomal compartments results in selective delivery of the hydrolases to the lysosome (L) (the 'kiss-and-run' theory[77]). TIP47/Rab9 prevent the MPRs from reaching the lysosomes, in which they would otherwise be degraded. The return pathway from the early endosomal compartment to the Golgi is probably mediated by PACS-1-assisted packaging into AP1-containing clathrin-coated vesicles (CCVs), whereas that from the late endosomal (LE) compartments is mediated by TIP47 and Rab9. Some of the MPRs go to the cell surface either from early or late endosomes through the recycling endosome (RE), or from proximal TGN cisternae as a consequence of mis-sorting. The cell-surface receptors are internalized in AP2 CCVs and delivered back to the endosomes.

Destination endosomes

After budding of the CCVs, the clathrin is released by HSC70, which is acting in conjunction with AUXILIN[62,63], but AP1 release requires a further unidentified cytoplasmic protein[64]. Time-lapse imaging has shown[59] that the AP1 remains associated with the vesicles for an extended period. This might allow interaction of its β1 subunit with kinesin family 13a (KIF13A)[65], which could transport the MPR-containing vesicles to the cell periphery along microtubule tracks (FIG. 7).

Several reports indicate that most of the MPR-containing vesicles deliver their cargo to early endosomes[60,66,67], but the interpretation of some of these data has been questioned[68]. There is considerable evidence that RAB GTPASES and SNARES are involved in the process of vesicle docking and fusion, but no specific Rab proteins have been identified in the vesicles as yet. However, rabaptin5 — a member of the Rab family — has been shown to bind to the ear domains of GGAs[39], the γ-subunit of AP1 (REF 69) and also to early endosomes through interaction with bound Rab5 (REF. 70). Therefore, if AP1 (or GGAs) remains associated with transport vesicles until they reach the target, rabaptin5 might have a role in the docking of the vesicles. Clathrin-coated vesicles contain several SNAREs, and three of these (syntaxin 6, syntaxin 13 and vesicle-associated membrane protein 4 (VAMP-4)) have been localized to CCVs in the TGN by electron microscopy[71–74]. Furthermore,VAMP-4 binds directly to AP1 through a dileucine motif[75].

One or more of these vesicle SNAREs probably participates in mediating the fusion of the vesicle with the endosome after docking. Syntaxin 6 is a good candidate as it binds to early endosomal antigen 1 (EEA1), which is the Rab5 effector on early endosomes[76]. During the maturation of early to late endosomes, the hydrolases are released in the low-pH milieu and then packaged into lysosomes when the late endosomes fuse with these organelles[77] (FIG. 4). The MPRs, however, do not enter the lysosomes. In the case of the CD-MPR, its retention in late endosomes is mediated by a phenylalanine–tryptophan motif in the cytoplasmic tail[78]. This motif is recognized by the cargo selection protein TIP47, which is thought to retain the CD-MPR in a region of the endosome that is not involved in the fusion–fission process with lysosomes[79].

Return to the Golgi

TIP47 also functions in the recycling of the MPRs to the TGN[79]. In addition to binding the phenylalanine–tryptophan motif in the cytoplasmic tail of the CD-MPR, TIP47 interacts with a complex motif in the tail of the CI-MPR that involves residues 48–74 (REFS 79,80). It also binds Rab9•GTP, and this interaction increases the affinity of TIP47 for the CI-MPR by threefold[81]. This would be expected to enhance the ability of TIP47 to capture its cargo. Using green fluorescent protein (GFP) variants of Rab9 in living cells, Rab9-positive transport vesicles have been visualized leaving late endosomal compartments and fusing with the TGN[82]. Although it wasn't possible to detect TIP47 or MPRs in these vesicles for technical reasons, these proteins were probably present, because transport of MPRs from endosomes to the TGN depends on both Rab9 and TIP47 (REF. 83).

There is also evidence that AP1 and the cytosolic sorting protein PACS-1 are involved in MPR recycling[84–86]. PACS-1 binds to the acidic amino acids that are clustered at the carboxyl terminus of the CI-MPR cytoplasmic tail[86]. In cells that lack either AP1 or PACS-1, the MPRs accumulate in endosomes, which indicates that there is a role for these proteins in endosome-to-Golgi trafficking. The connection between AP1 and PACS-1 became clear when it was discovered that PACS-1 binds to AP1 as well as to the MPRs, providing a mechanism for connecting the cargo to the adaptor for packaging into CCVs in early endosomes[87]. This is similar to the role of the GGAs in presenting MPRs to AP1 for incorporation into AP1-CCVs at the TGN.

HSC70
(heat shock cognate protein of 70kDa). Proteins of this chaperone family are involved in a range of cellular processes, such as protein folding, translocation across membranes and the assembly or disassembly of protein complexes.

AUXILIN
A neuronal protein that contains a clathrin-binding site and a carboxy-terminal J-domain that interacts with and stimulates the dormant ATPase of the chaperone Hsc70.

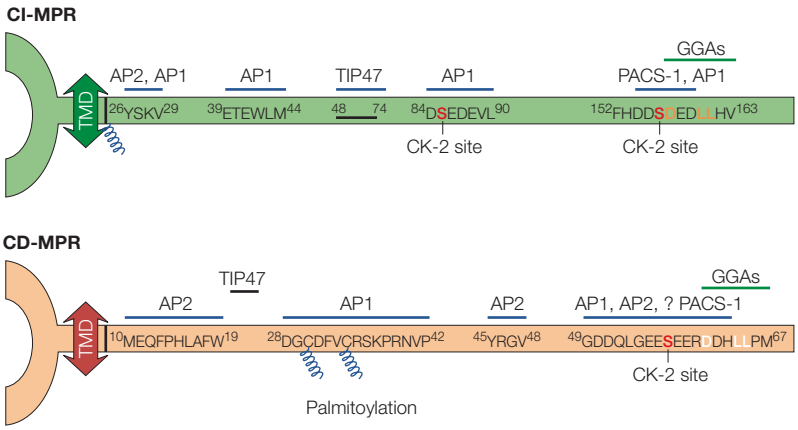

Figure 5 | **Sorting signals on MPR tails.** A schematic representation of the cytosolic tails of the mannose 6-phosphate receptors (MPRs), which shows the identified amino-acid sorting signals and their associated transport proteins. The bars indicate the residues that are important for the specific binding partner. The residues are numbered from 1 to 163 in the cation-independent (CI)-MPR, and from 1 to 67 in the cation-dependent (CD)-MPR, starting from the transmembrane domain (TMD). The casein kinase 2 (CK-2) sites and palmitoylation sites are marked at the specific residues. GGA, Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding protein; PACS-1, phosphofurin acidic cluster-sorting protein; TIP47, tail-interacting protein, 47 kDa.

These data indicate that PACS-1/AP1 mediates receptor recycling from early endosomes, whereas TIP47/Rab9 recycles receptors from late endosomes. However, the relative contribution of these two pathways to the total MPR trafficking remains to be determined. It has also been reported that mutation of the dileucine pair near the carboxyl terminus of the CD-MPR causes a shift in receptor distribution from the TGN to early endosomes, indicating that there is a role for the dileucine motif in a sorting event that is required for MPRs to return to the Golgi[88]. The binding factor that recognizes the dileucine motif for endosome-to-TGN trafficking is unknown as *in vitro* analysis indicates that the carboxy-terminal dileucine motif has little effect on AP1, TIP47 and PACS-1 binding[31,32,47,80,86]. As GGA2 has been found in endosomes as well as the TGN, it (or another GGA) is a candidate for participating in this sorting step[89].

Traffic to the cell surface

The cell's surface environment is slightly alkaline (pH 7.4), and under these conditions the CI-MPR, but not the CD-MPR, binds a diverse group of M6P-containing ligands and non-glycosylated polypeptides, including IGF-II (TABLE 1). There are several ways by which the MPRs could reach the cell surface: mis-sorting at the TGN, with the MPRs exiting the Golgi through transport carriers derived from stacks C5/6 and being destined for the plasma membrane; and/or recycling from early[88] or late endosomes[83].

Once at the cell surface, the MPRs are internalized rapidly. The internalization of the CI-MPR is mediated almost exclusively by its YSKV motif[90], whereas the CD-MPR contains three separate internalization sequences: a phenylalanine-containing sequence; a tyrosine-based motif; and the carboxy-terminal region that contains the acidic amino-acid cluster sequence[91,92] (FIG. 5). Each of these motifs contributes equally to internalization. Direct binding studies with AP2, the plasma membrane adaptor and its μ2 subunit showed interaction of all three motifs, although the results varied depending on the technique used[32,93]. As with AP1, the carboxy-terminal dileucine residues of the MPRs are not required for binding to AP2. This is curious as there is considerable evidence that dileucine motifs present in other proteins do bind to AP1 and AP2 (REFS 50,51).

The rate of internalization of the CI-MPR is increased considerably on binding of the multivalent ligand β-glucuronidase[13]. This might be the consequence of a conformational change that results in a more optimal presentation of the internalization signal in the cytoplasmic tail.

The CI-MPR regulates cell growth and motility

A growing body of evidence indicates that the CI-MPR has a role in regulating cell growth and motility. The CI-MPR binds and internalizes IGF-II at the cell surface, thereby modulating extracytoplasmic levels of this mitogenic polypeptide hormone. The importance of IGF-II clearance by the CI-MPR was shown by the finding that CI-MPR-deficient mice have increased levels of circulating IGF-II and enlarged organs. These mice die at, or soon after, birth owing to heart failure secondary to cardiac hyperplasia[94–96]. The introduction of an *IGF-II* null allele (double-knockout mice) rescued the CI-MPR mutant mice from perinatal lethality, which is consistent with IGF-II clearance being an essential function of the CI-MPR[94,95].

As well as its established role in IGF-II clearance, several reports[2,97–104] indicate that the CI-MPR also functions in IGF-II signalling. This is controversial, however, as the cytoplasmic domain of the receptor lacks any known enzymatic activity, and the mitogenic activity of IGF-II has been shown to be mediated by the IGF-I receptor[1] and the recently identified insulin-receptor isoform A[105] in several instances. To try to achieve receptor specificity, more recent studies have used IGF-II analogues that bind to the CI-MPR, but not to the IGF-I receptor or IGF-binding proteins. The CI-MPR-specific IGF-II analogues induce chemotaxis of extravillous trophoblast cells[99], endothelial cells[100] and rhabdomyosarcoma cells[101], indicating that CI-MPR has a role in regulating cell motility.

Several reports have indicated that this signalling occurs through a G-protein-coupled pathway that leads to activation of mitogen-activated protein kinase (MAPK)[97,99,100,104]. However, a very careful study has presented evidence against this conclusion[106]. In addition to IGF-II, the placental angiogenic hormone proliferin, an M6P-containing protein, has also been reported to initiate chemotaxis in endothelial cells on binding to the CI-MPR[100]. Furthermore, IGF-II and M6P-containing glycoproteins that are produced by SERTOLI CELLS seem to modulate gene expression in spermatogonia by binding to the CI-MPR at the cell surface[102]. And in the immune system, binding of the T-cell surface molecule CD26/dipeptidyl peptidase IV to the

KIF13A
(kinesin family 13A). A novel plus-end-directed microtubule-dependent motor protein that associates with mannose 6-phosphate receptor (MPR)-containing carriers through the β1-subunit of AP1.

RAB PROTEINS
Monomeric small GTPases which, along with their effectors, mediate the first specific event during membrane fusion; that is, tethering of an incoming vesicle to the correct target organelle.

SNARES
(soluble *N*-ethyl-maleimide-sensitive (NSF) attachment protein receptors). Proteins that are implicated in mediating most intracellular membrane-fusion events by interacting with each other to generate the driving force needed to fuse lipid bilayers.

TIP47
(tail-interacting protein, 47 kDa). A novel hydrophilic, cytosolic protein of 47 kDa that binds directly to cytoplasmic tails of both the cation-independent and cation-dependent MPRs and is involved in sorting at the endosomes.

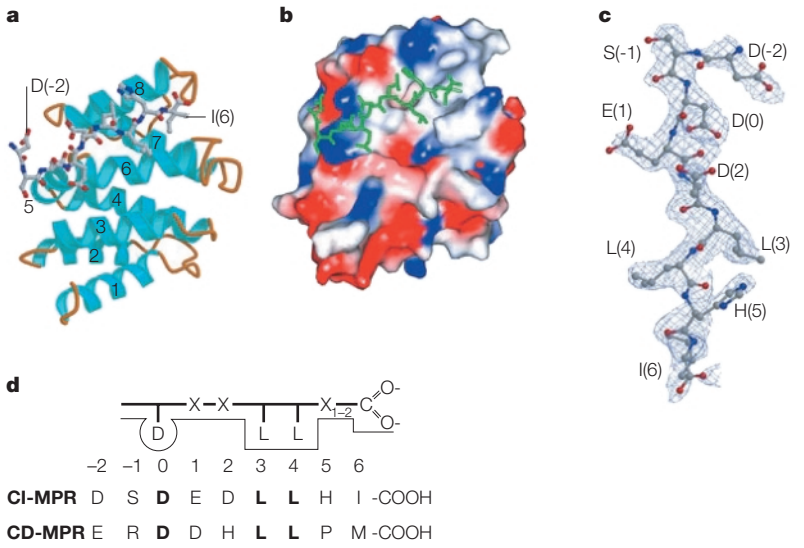

Figure 6 | **The structure of the GGA3 VHS domain bound to its ligand. a** | The structure of the VHS domain of Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding protein 3 (GGA3) with the cation-independent mannose 6-phosphate (M6P)/insulin-like growth factor II (IGF-II) receptor (CI-MPR) acidic cluster dileucine motif (AC–LL; 'ball-and-stick' model) bound between helices 6 and 8 (REF. 41). The first and last visible residues of the CI-MPR peptide are labelled. **b** | The molecular surface of the VHS domain, coloured by electrostatic potential. Saturated blue and red areas are at +10*kT* and –10*kT* respectively. The CI-MPR peptide is shown in green. **c** | The contour of the CI-MPR sorting signal. **d** | The primary determinants of peptide binding to the VHS domain (where X is any amino acid). Aligned below the primary determinants are the CI-MPR and cation-dependent (CD)-MPR AC–LL sequences. The crucial aspartate is designated the '0' reference point. Modifed with permission from REF. 41 *Nature* © Macmillan Magazines Ltd (2002).

CI-MPR through M6P residues facilitates T-cell activation[103], whereas soluble CD26 released from T cells enhances transendothelial cell migration after binding to the CI-MPR[107]. The interpretation of these reports is complicated by the fact that many different cell types and assays have been used.

In addition, CI-MPR is thought to facilitate the activation of the TGF-β1 precursor, the proform of a hormone that has many effects on cell growth and differentiation. Latent TGF-β1, which is an M6P-containing molecule[108], is secreted by cells and then stored in the extracellular matrix as an inactive complex with latency-associated peptide. A major mechanism of TGF-β1 activation is through the matrix glycoprotein thrombospondin-1 (REF. 109). Several groups have reported another mechanism that involves binding of the TGF-β1 precursor to the cell-surface CI-MPR[110–114]. On the basis of results from *in vitro* assays, Godar *et al.*[112] proposed an elegant model whereby the CI-MPR forms a complex with the urokinase (plasminogen activator) receptor (uPAR) through a binding site on the extracellular domain 1 of the CI-MPR receptor. Urokinase bound to uPAR then converts plasminogen, which is also bound to domain 1 (REF. 115), to plasmin, which mediates the release of active TGF-β1 from its receptor-bound latent form. However, these authors have also reported that in intact cells the CI-MPR downregulates uPAR-mediated plasminogen activation[115], possibly through the targeting of surface uPAR to lysosomes[116]. This apparently contradictory finding could be explained if the local generation of plasmin by cell-surface CI-MPR, rather than the total plasmin formation, is the crucial factor in the regulated formation of active TGF-β1. Furthermore, at this point, the contribution of the CI-MPR pathway for latent TGF-β1 activation relative to the other known mechanisms of activation is unknown.

The CI-MPR has also been reported to bind retinoic acid at a site distinct from those for M6P and IGF-II, which results in changes in cell shape, reduced spreading, apoptosis and growth inhibition[117]. These effects were not observed in cells that lacked the CI-MPR and were not prevented by a potent antagonist of the retinoic-acid nuclear receptor (RAR), which indicates that the effects are mediated by the CI-MPR rather than RARs. These findings indicate that retinoic acid might induce growth inhibition and apoptosis independently of the RARs by altering the behaviour of the CI-MPR.

CI-MPR as a putative tumour suppressor

The ability of the CI-MPR to modulate local levels of the mitogen IGF-II, to facilitate activation of the growth inhibitor TGF-β1 and to regulate trafficking of lysosomal enzymes indicates that it could be a tumour suppressor. The finding that overexpression of the CI-MPR results in growth inhibition both *in vitro* and *in vivo* is consistent with this idea[118,119]. Further support for this hypothesis comes from studies of human cancers, which indicate that loss of CI-MPR function is associated with progression of tumorigenesis.

Frequent LOSS OF HETEROZYGOSITY (LOH) at the *CI-MPR* locus has been reported in human liver[120–122], breast[123,124], lung[125], ovarian[126] and adrenocortical[127] cancers. This has been observed most commonly in the liver, in which about 60% of dysplastic lesions and hepatocellular carcinomas in patients with and without hepatitis C virus infection show LOH. In some of these cases, the M6P- and IGF-II-binding domains of the remaining allele were screened for mutations, and a number were found[120–123,126,128]. These include single-base deletions in the polydeoxyguanosine (poly G) region of domain 9, which is a target of MICROSATELLITE INSTABILITY in replication/repair error-positive tumours. These cause a frameshift that results in the synthesis of a truncated receptor that lacks the transmembrane domain and is presumably secreted as a soluble protein or is degraded. Four of the five missense mutations that have been examined disrupted M6P- and/or IGF-II binding properties, which supports the proposal that loss of normal CI-MPR function contributes to carcinogenesis[129,130].

The LOH at the *CI-MPR* locus is an early event in liver carcinogenesis. Examination of phenotypically normal hepatocytes in cirrhotic nodules that are adjacent to dysplastic lesions and hepatocellular carcinomas showed loss of the same allele of the *CI-MPR* as observed in the tumour[121,122]. This shows that inactivation of the *CI-MPR* allele occurs before there are any observable phenotypic changes in the hepatocyte. Although it is not known whether inactivation of a

PACS-1
(phosphofurin acidic cluster-sorting protein). A cytosolic linker molecule that connects proteins such as furin, HIV virus type-1 (HIV-1) Nef and the CI-MPR, through their acidic-cluster sorting motifs, to adaptors (AP1 and AP3, but not AP2).

SERTOLI CELL
A cell that is located in the male gonads and provides nourishment to sperm.

LOSS OF HETEROZYGOSITY
A loss of either the maternal or paternal allele of a gene. This is often a molecular marker of a tumour-suppressor gene locus.

MICROSATELLITE INSTABILITY
(MSI). Alterations of the length of simple repetitive genomic sequences. In tumours it is an indication that there have probably been mutations in genes encoding proteins that are involved in DNA repair.

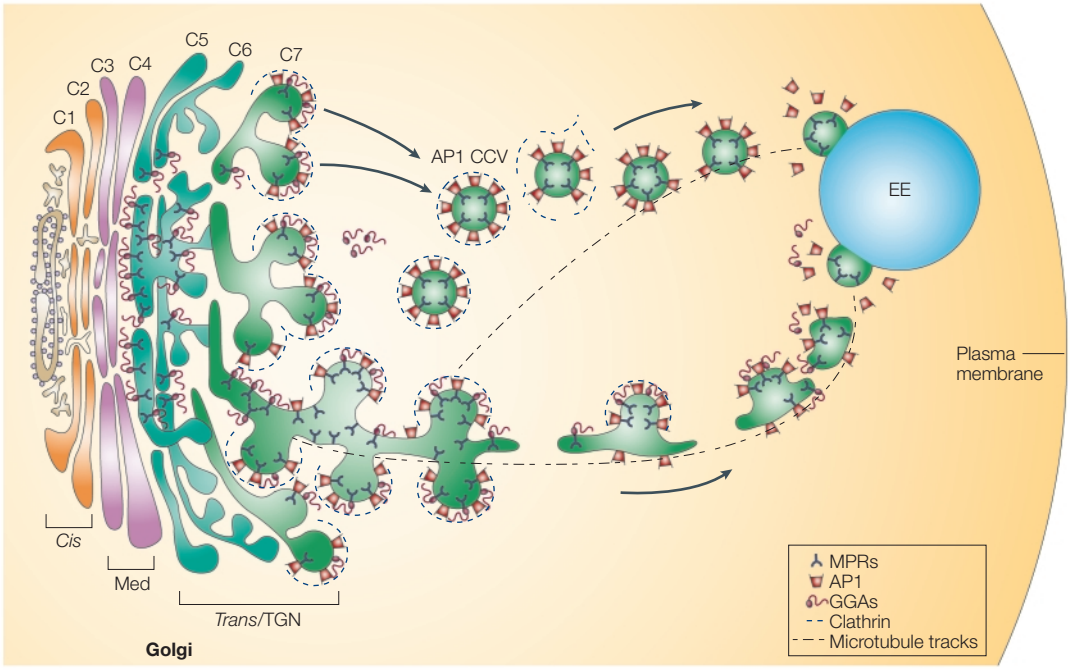

Figure 7 | **Architecture of the *trans*-Golgi network and its relevance to MPR trafficking.** Golgi stacks are numbered C1 to C7. The last two or three stacks and their tubulo-vesicular extensions form the *trans*-Golgi/*trans*-Golgi network (*Trans*/TGN). Clathrin-coated buds vesiculate exclusively from the C7 cisterna[55,56]. The penultimate two cisternae contribute tubules and buds that are non-clathrin coated and thought to be destined for the plasma membrane. The C5 stack projects tubules in either direction and functions as a transition zone between the medial Golgi and *trans*-Golgi. GGA proteins are distributed equally between clathrin-coated and smooth membranes, which raises the possibility that they encounter the mannose 6-phosphate receptors (MPRs) earlier than the AP1 adaptors, which are concentrated on clathrin-coated membranes. Being favourably positioned, GGAs could be involved in the early sorting of MPRs, retaining them within the TGN until they reach the C7 cisterna. Clathrin-coated buds are formed at the tips and along the length of the TGN-derived tubules. GGAs and MPRs colocalize in these buds along with AP1. The GGAs transfer the bound MPRs to AP1 and then return to the cytosol. After budding of the AP1 clathrin-coated vesicles (CCVs), the clathrin is released, while the AP1 remains for 'post-budding' functions. This is followed by adaptor release before fusion with endosomal membranes. The TGN-derived tubules could also carry MPRs to their endosomal destination. The tips of these tubules detach after a certain distance and form tubulo-vesicular carriers that might fuse directly with endosomes[38,60]. The tubules, their detached segments and the clathrin-uncoated vesicles could be guided to their destination by the kinesin family 13A (KIF13A) on microtubule tracks[65]. *Cis*, *cis*-Golgi; EE, early endosome; Med, medial Golgi; RER, rough endoplasmic reticulum.

single *CI-MPR* allele provides a growth advantage to the mutated hepatocytes, the clonal growth of these cells provides an enlarged population of pre-malignant cells in which only a single genetic 'hit' is required to inactivate the CI-MPR tumour-suppressor function completely. Similarly, the inactivation of the *CI-MPR* gene is also an early event in breast cancer[124].

More to come

Despite the advances in understanding the MPRs, many interesting questions remain. So far, only 4 of the 15 repeating segments of the receptor have been implicated in ligand binding. Although some of the other segments might interact with retinoic acid, most of them would still have no assigned function. Do these have solely structural roles, perhaps providing crucial spacing between ligand-binding domains, or could they interact with further ligands?

With the successful elucidation of the structures of the M6P-binding domain of the CD-MPR and the IGF-II binding domain of the CI-MPR, it seems probable that the structures of the M6P-binding domains of the CI-MPR will also be solved. What has lagged behind is the analysis of the structures of the cytoplasmic tails of the receptors. With an array of sorting signals, their conformations might be modulated by phosphorylation and/or palmitoylation. This could provide a mechanism for regulating the interaction of the sorting signals with their binding partners. One example of such a conformation-dependent interaction is the binding of TIP47 to the tail of the CI-MPR[80]. In this respect, an interesting observation is the conspicuous stretch of prolines in the cytosolic tail of the CI-MPR and the conserved prolines in the CD-MPR tail. Further studies are needed to determine whether these prolines are responsible for twists in the tails.

Recent developments in the MPR trafficking story have implicated the GGAs as having a key role in sorting at the TGN. The phosphoregulation of MPR–GGA–AP1 interactions is an interesting model that needs to be probed further. For example, when and where does the phosphorylation of MPRs and GGAs(1/3) occur? As GGA2 is not phosphorylated, does it have a special role in MPR trafficking? If so, how is it

Table 1 | **Selected ligands for the cation-independent M6P/IGF-II receptor (CI-MPR)**

Ligands	Consequences of binding to the CI-MPR	References
M6P-containing ligands		
Lysosomal enzymes	Endocytosis and/or targeting to lysosomes	1–4
TGF-β1 precursor	Proteolytic activation at the cell surface	108–115
Proliferin	Endothelial-cell migration and angiogenesis	100
Granzyme B	Internalization and rapid induction of apoptosis	134
Non-M6P-containing ligands		
IGF-II	Endocytosis and degradation in lysosomes	
Retinoic acid	Growth inhibition and/or induction of apoptosis	117
uPAR	Part of the cell-surface-assembled complex that activates latent TGF-β1. Endocytosis and degradation in lysosomes	112,115,116
Plasminogen	Conversion to plasmin, which can activate TGF-β1 from its latent proform	112,115

For a complete list of the known ligands, see REF. 1. CI-MPR, cation-independent M6P/IGF-II receptor; IGF-II, insulin-like growth factor II; M6P, mannose 6-phosphate; TGF-β1, transforming growth factor-β1; uPAR, urokinase-type (plasminogen activator) receptor.

regulated? The recent report of the crystal structure of the trunk of AP2 has shown that the binding site for YXXϕ endocytic motifs on the μ2 subunit is buried, and is probably exposed by a conformational change that is triggered by phosphorylation of μ2 (REF. 131). Consistent with this interpretation, phosphorylation of μ2 strongly increases binding to endocytic signals[132]. It will be important to determine whether AP1 shows similar phosphorylation-dependent conformational changes in its μ1 subunit, and what effects this might have on ligand binding.

Live video-microscopy using fluorescently tagged proteins has shown the dynamic nature of the sorting process at the TGN. It will be very informative to apply this approach to cells expressing tagged GGAs and AP1. This will bear directly on the issue of whether the GGAs and AP1 exit the TGN on the same or different tubules/vesicles. Another challenge is to ascertain the relative roles of PACS-1/AP1 versus TIP47/Rab9 in mediating the return of the MPRs from endosomes to the Golgi. The availability of techniques such as RNA interference and gene disruption for knocking out these proteins should help to clarify this issue.

Finally, with respect to the role of the CI-MPR as a putative tumour suppressor, the relationship between the genetic alterations and the pathogenesis, progression, metastatic potential and prognosis of the tumours needs to be clarified[133]. For instance, does the behaviour of the tumour vary depending on whether there is a point mutation in the M6P-binding domain versus the IGF-II-binding domain? Considering the difficulty of finding large numbers of patients with the different mutations, it will be difficult to address this issue.

With so many questions outstanding, it seems certain that the MPRs will continue to fascinate investigators for many years to come.

1. Dahms, N. M. & Hancock, M. K. P-type lectins. *Biochim. Biophys. Acta*. **1572**, 317–340 (2002).
2. Kornfeld, S. Structure and function of the mannose 6-phosphate/insulin like growth factor II receptors. *Annu. Rev. Biochem*. **61**, 307–330 (1992).
3. Hille-Rehfeld, A. Mannose 6-phosphate receptors in sorting and transport of lysosomal enzymes. *Biochim. Biophys. Acta.* **1241**, 177–194 (1995).
4. Le Borgne, R. & Hoflack, B. Protein transport from the secretory to the endocytic pathway in mammalian cells. *Biochim. Biophys. Acta.* **1404**, 195–209 (1998).
5. Lobel, P., Dahms, N. M. & Kornfeld, S. Cloning and sequence analysis of the cation-independent mannose 6-phosphate receptor. *J. Biol. Chem.* **263**, 2563–2570 (1988).
6. Hancock, M. K., Haskins, D. J., Sun, G. & Dahms, N. M. Identification of residues essential for carbohydrate recognition by the insulin-like growth factor II/mannose 6-phosphate receptor. *J. Biol. Chem.* **277**, 11255–11264 (2002).
7. Marron-Terada, P. G., Hancock, D. J., Haskins, D. J. & Dahms, N. M. Recognition of *Dictyostelium discoideum* lysosomal enzymes is conferred by the amino-terminal carbohydrate binding site of the insulin-like growth factor II/mannose 6-phosphate receptor. *Biochemistry* **39**, 2243–2253 (2000).
8. Schmidt, B., Kiecke-Siemsen, C., Waheed, A., Braulke, T. & von Figura, K. Localization of the insulin-like growth factor II binding site to amino acids 1508–1566 in repeat 11 of the mannose 6-phosphate/insulin-like growth factor II receptor. *J. Biol. Chem.* **270**, 14975–14982 (1995).
9. Garmroudi, F., Devi, G., Slentz, D. H., Schaffer, B. S. & MacDonald, R. G. Truncated forms of the insulin-like growth factor II (IGF-II)/mannose 6-phosphate receptor encompassing the IGF-II binding site: characterization of a point mutation that abolishes IGF-II binding. *Mol. Endocrinol.* **10**, 642–651 (1996).
References 6–9 characterize the carbohydrate-binding domain and the IGF-II-binding site on the CI-MPR.
10. Meresse, S., Ludwig, T., Frank, R. & Hoflack, B. Phosphorylation of the cytoplasmic domain of the bovine cation-independent mannose 6-phosphate receptor. Serines 2421 and 2492 are the targets of a casein kinase II associated to the Golgi-derived HAI adaptor complex. *J. Biol. Chem.* **265**, 18833–18842 (1990).
11. Rosorius, O. *et al*. Characterization of phosphorylation sites in the cytoplasmic domain of the 300 kDa mannose 6-phosphate receptor. *Biochem. J.* **292**, 833–838 (1993).
12. Schweizer, A., Kornfeld, S. & Rohrer, J. Cysteine 34 of the cytoplasmic tail of the cation-dependent mannose 6-phosphate receptor is reversibly palmitoylated and required for normal trafficking and lysosomal enzyme sorting. *J. Cell Biol.* **132**, 577–584 (1996).
13. York, S. J., Arneson, L. S., Gregory, W. T., Dahms, N. M. & Kornfeld, S. The rate of internalization of the mannose 6-phosphate/insulin-like growth factor II receptor is enhanced by multivalent ligand binding. *J. Biol. Chem.* **274**, 1164–1171 (1999).
14. Byrd, J. C. & MacDonald, R. G. Mechanisms for high affinity mannose 6-phosphate ligand binding to the insulin-like growth factor II/mannose 6-phosphate receptor. Negative cooperativity and receptor oligomerization. *J. Biol. Chem.* **275**, 18638–18646 (2000).
15. Byrd, J. C., Park, J. H., Schaffer, B. S., Garmroudi, F. & MacDonald, R. G. Dimerization of the insulin-like growth factor II/mannose 6-phosphate receptor. *J. Biol. Chem.* **275**, 18647–18656 (2000).
16. Roberts, D. L., Weix, D. J., Dahms, N. M. & Kim, J.-J. P. Molecular basis of lysosomal enzyme recognition: three-dimensional structure of the cation-dependent mannose 6-phosphate receptor. *Cell* **93**, 639–648 (1998).
17. Olson, L. J., Zhang, J., Lee, Y. C., Dahms, N. M. & Kim, J.-J. P. Structural basis for recognition of phosphorylated high mannose oligosaccharides by the cation-dependent mannose 6-phosphate receptor. *J. Biol. Chem.* **274**, 29889–29896 (1999).
18. Olson, L. J., Zhang, J., Dahms, N. M. & Kim, J.-J. P. Twists and turns of the CD-MPR: ligand-bound versus ligand-free receptor. *J. Biol. Chem.* **277**, 10156–10161 (2002).
References 16–18 provide useful insights into the structural basis for M6P-containing ligand recognition by the CD-MPR.
19. Brown, J. *et al*. Structure of a functional IGF2R fragment determined from the anomalous scattering of sulfur. *EMBO J.* **21**, 1054–1062 (2002).
This work presents a high-resolution crystal structure of the eleventh domain of the CI-MPR that functions as the IGF-II-binding domain.

20. Zeslawski, W. *et al*. The interaction of insulin-like growth factor-I with the N-terminal domain of IGFBP-5. *EMBO J.* **20**, 3638–3644 (2001).
21. Devi, G. R., Byrd, J. C., Slentz, D. H. & MacDonald, R. G. An insulin-like growth factor II (IGF-II) affinity-enhancing domain localized within extracytoplasmic repeat 13 of the IGF-II/mannose 6-phosphate receptor. *Mol. Endocrinol.* **12**, 1661–1672 (1998).
22. Linnell, J., Groeger, G. & Hassan, A. B. Real time kinetics of insulin-like growth factor II (IGF-II) interaction with the IGF-II/mannose 6-phosphate receptor: the effects of domain 13 and pH. *J. Biol. Chem.* **276**, 23986–23991 (2001).
23. Rohrer, J. & Kornfeld, R. Lysosomal hydrolase mannose 6-phosphate uncovering enzyme resides in the *trans*-Golgi network. *Mol. Biol. Cell* **12**, 1623–1631 (2001).
24. Klumperman, J. *et al*. Differences in the endosomal distributions of the two mannose 6-phosphate receptors. *J. Cell Biol.* **121**, 997–1010 (1993).
This report established that the two receptors exit the *trans*-Golgi network through AP1-positive clathrin-coated buds and vesicles.
25. Campbell, C. H. & Rome, L. H. Coated vesicles from rat liver and calf brain contain lysosomal enzymes bound to mannose 6-phosphate receptors. *J. Biol. Chem.* **258**, 13347–13352 (1983).
26. Schulze-Lohoff, E., Hasilik, A. & von Figura, K. Cathepsin D precursors in clathrin-coated organelles from human fibroblasts. *J. Cell Biol.* **101**, 824–829 (1985).
References 25 and 26 showed that clathrin-coated vesicles function as transport carriers for lysosomal enzymes *en route* from the Golgi to the endosomal–lysosomal compartments.
27. Johnson, K. F. & Kornfeld, S. The cytoplasmic tail of the mannose 6-phosphate/insulin-like growth factor-II receptor has two signals for lysosomal enzyme sorting in the Golgi. *J. Cell Biol.* **119**, 249–257 (1992).
28. Johnson, K. F. & Kornfeld, S. A His–Leu–Leu sequence near the carboxyl terminus of the cytoplasmic domain of the cation-dependent mannose 6-phosphate receptor is necessary for the lysosomal enzyme sorting function. *J. Biol. Chem.* **267**, 17110–17115 (1992).
29. Chen, H. J., Remmler, J., Delaney, J. C., Messner, D. J. & Lobel, P. Mutational analysis of the cation-independent mannose 6-phosphate/insulin-like growth factor II receptor. A consensus casein kinase II site followed by 2 leucines near the carboxyl terminus is important for intracellular targeting of lysosomal enzymes. *J. Biol. Chem.* **268**, 22338–22346 (1993).
30. Chen, H. J., Yuan, J. & Lobel, P. Systematic mutational analysis of the cation-independent mannose 6-phosphate/insulin-like growth factor II receptor cytoplasmic domain. An acidic cluster containing a key aspartate is important for function in lysosomal enzyme sorting. *J. Biol. Chem.* **272**, 7003–7012 (1997).
References 27–30 establish the role of the AC-LL signal in trafficking of the mannose 6-phosphate receptors from the *trans*-Golgi network to endosomes.
31. Mauxion, F., Le Borgne, R., Munier-Lehmann, H. & Hoflack, B. A casein kinase II phosphorylation site in the cytoplasmic domain of the cation-dependent mannose 6-phosphate receptor determines the high affinity interaction of the AP-1 Golgi assembly proteins with membranes. *J. Biol. Chem.* **271**, 2171–2178 (1996).
32. Honing S., Sosa M., Hille-Rehfeld, A. & von Figura, K. The 46-kDa mannose 6-phosphate receptor contains multiple binding sites for clathrin adaptors. *J. Biol. Chem.* **272**, 19884–19890 (1997).
33. Poussu A., Lohi, O. & Lehto, V. P. Vear, a novel Golgi-associated protein with VHS and γ-adaptin 'ear' domains. *J. Biol. Chem.* **275**, 7176–7183 (2000).
34. Hirst, J. *et al*. A family of proteins with γ-adaptin and VHS domains that facilitate trafficking between the *trans*-Golgi network and the vacuole/lysosome. *J. Cell Biol.* **149**, 67–80 (2000).
35. Dell'Angelica, E. C. *et al*. GGAs: A family of ADP ribosylation factor-binding proteins related to adaptors and associated with the Golgi complex. *J. Cell Biol.* **149**, 81–94 (2000).
36. Boman, A. L., Zhang, C., Zhu, X. & Kahn, R. A. A family of ADP-ribosylation factor effectors that can alter membrane transport through the *trans*-Golgi. *Mol. Biol. Cell* **11**, 1241–1255 (2000).
37. Takatsu, H., Yoshino, K. & Nakayama, K. Adaptor γ ear homology domain conserved in γ-adaptin and GGA proteins that interact with γ-synergin. *Biochem. Biophys. Res. Commun.* **271**, 719–725 (2000).
References 33–37 cite the five groups that simultaneously and independently discovered the GGA family of adaptors.
38. Puertollano, R., Aguilar, R. C., Gorshkova, I., Crouch, R. J. & Bonifacino, J. S. Sorting of mannose 6-phosphate receptors mediated by the GGAs. *Science* **292**, 1712–1716 (2001).
39. Zhu, Y., Doray, B., Poussu, A., Lehto, V. P. & Kornfeld, S. Binding of GGA2 to the lysosomal enzyme sorting motif of the mannose 6-phosphate receptor. *Science* **292**, 1716–1718 (2001).
40. Takatsu, H., Katoh, Y., Shiba, Y. & Nakayama, K. Golgi-localizing, γ-adaptin ear homology domain, ADP-ribosylation factor-binding (GGA) proteins interact with acidic dileucine sequences within the cytoplasmic domains of sorting receptors through their Vps27p/Hrs/STAM (VHS) domains. *J. Biol. Chem.* **276**, 28541–28545 (2001).
References 38–40 implicated the GGAs in sorting of mannose 6-phosphate receptors at the *trans*-Golgi network.
41. Misra, S., Puertollano, R., Kato, Y., Bonifacino, J. S. & Hurley, J. H. Structural basis for acidic-cluster-dileucine sorting-signal recognition by VHS domains. *Nature* **415**, 933–937 (2002).
42. Shiba, T. *et al*. Structural basis for recognition of acidic-cluster dileucine sequence by GGA1. *Nature* **415**, 937–941 (2002).
References 41 and 42 demonstrate the structural basis for the recognition of the AC–LL signal in the cytoplasmic tail of the MPR by the VHS domain of the GGA protein.
43. Jacobsen, L. *et al*. The sorLA cytoplasmic domain interacts with GGA1 and -2 and defines minimum requirements for GGA binding. *FEBS Lett.* **511**, 155–158 (2002).
44. Doray, B., Bruns, K., Ghosh, P. & Kornfeld, S. Interaction of the cation-dependent mannose 6-phosphate receptor with GGA proteins. *J. Biol. Chem.* **277**, 18477–18482 (2002).
45. Puertollano, R., Randazzo, P. A., Presley, J. F., Hartnell, L. M. & Bonifacino, J. S. The GGAs promote ARF-dependent recruitment of clathrin to the TGN. *Cell*. **105**, 93–102 (2001).
46. Dittie, A. S., Thomas, L., Thomas, G. & Tooze, S. A. Interaction of furin in immature secretory granules from neuroendocrine cells with the AP-1 adaptor complex is modulated by casein kinase II phosphorylation. *EMBO J.* **16**, 4859–4870 (1997).
47. Le Borgne, R., Schmidt, A., Mauxion, F., Griffiths, G. & Hoflack B. Binding of AP-1 Golgi adaptors to membranes requires phosphorylated cytoplasmic domains of the mannose 6-phosphate/insulin-like growth factor II receptor. *J. Biol. Chem.* **268**, 22552–22556 (1993).
48. Ohno, H. *et al*. The medium subunits of adaptor complexes recognize distinct but overlapping sets of tyrosine-based sorting signals. *J. Biol. Chem.* **273**, 25915–25921 (1998).
49. Owen, D. J. & Evans, P. R. A structural explanation for the recognition of tyrosine-based endocytic signals. *Science.* **282**, 1327–1332 (1998).
50. Bremnes, T., Lauvrak, V., Lindqvist, B. & Bakke, O. A region from the medium chain adaptor subunit (μ) recognizes leucine- and tyrosine-based sorting signals. *J. Biol. Chem.* **273**, 8638–8645 (1998).
51. Rapoport, I., Chen, Y. C., Cupers, P., Shoelson, S. E. & Kirchhausen, T. Dileucine-based sorting signals bind to the β chain of AP-1 at a site distinct and regulated differently from the tyrosine-based motif-binding site. *EMBO J.* **17**, 2148–2155 (1998).
52. Black, M. W. & Pelham, H. R. A selective transport route from Golgi to late endosomes that requires the yeast GGA proteins. *J. Cell Biol.* **151**, 587–600 (2000).
53. Costaguta, G., Stefan, C. J., Bensen, E. S., Emr S. D. & Payne, G. S. Yeast GGA coat proteins function with clathrin in Golgi to endosome transport. *Mol. Biol. Cell* **12**. 1885–1896 (2001).
54. Hirst, J., Lindsay, M. R. & Robinson, M. S. GGAs: Roles of the different domains and comparison with AP-1 and clathrin. *Mol. Biol. Cell* **12**, 3573–3588 (2001).
55. Ladinsky, M. S., Mastronarde, D. N., McIntosh, J. R., Howell, K. E. & Staehelin, L. A. Golgi structure in three dimensions: functional insights from the normal rat kidney cell. *J. Cell Biol.* **144**, 1135–1149 (1999).
56. Marsh, B. J., Mastronarde, D. N., Buttle, K. F., Howell, K. E. & McIntosh, J. R. Organellar relationships in the Golgi region of the pancreatic β-cell line, HIT-T15, visualized by high resolution electron tomography. *Proc. Natl Acad. Sci. USA* **98**, 2399–2406 (2001).
57. Doray, B., Ghosh, P., Griffith, J., Geuze, H. & Kornfeld, S. Cooperation of GGAs and AP-1 in packaging MPRs at the *trans*-Golgi network. *Science*, **297**, 1700–1703 (2002).
This work showed that GGAs and AP1 colocalize within clathrin-coated buds and vesicles at the *trans*-Golgi network (TGN) of mammalian cells, which indicates a cooperative model for AP1 and GGAs in trafficking of MPRs from the TGN to endosomes, as opposed to the independent pathways proposed in yeast.
58. Doray, B., Bruns, K., Ghosh, P. & Kornfeld, S. A. Autoinhibition of the ligand-binding site of GGA1/3 VHS domains by an internal acidic cluster-dileucine motif. *Proc. Natl Acad. Sci. USA* **99**, 8072–8077 (2002).
59. Huang, F., Nesterov, A., Carter, R. E. & Sorkin, A. Trafficking of yellow-fluorescent-protein-tagged μ1 subunit of clathrin adaptor AP-1 complex in living cells. *Traffic* **2**, 345–357 (2001).
The first evidence of AP1 in anterograde trafficking of MPRs using live-cell imaging techniques.
60. Waguri, S. *et al*. Visualization of TGN to endosomes trafficking through fluorescently labeled MPR and AP-1 in living cells. *Mol. Biol. Cell* **14**, 142–155 (2003).
61. Le Borgne, R. & Hoflack, B. Mannose 6-phosphate receptors regulate the formation of clathrin-coated vesicles in the TGN. *J. Cell Biol.* **137**, 335–345 (1997).
62. Umeda, A., Meyerholz, A. & Ungewickell, E. Identification of the universal cofactor (auxilin 2) in clathrin coat dissociation. *Eur. J. Cell Biol.* **79**, 336–342 (2000).
63. Greener, T., Zhao, X., Nojima, H., Eisenberg, E. & Greene, L. E. Role of cyclin G-associated kinase in uncoating clathrin-coated vesicles from non-neuronal cells. *J. Biol. Chem*. **275**, 1365–1370 (2000).
64. Hannan, L. A., Newmyer, S. L. & Schmid, S. L. ATP- and cytosol-dependent release of adaptor proteins from clathrin-coated vesicles: a dual role for Hsc70. *Mol. Biol. Cell* **9**, 2217–2229 (1998).
65. Nakagawa, T. *et al*. A novel motor, KIF13A, transports mannose 6-phosphate receptor to plasma membrane through direct interaction with AP-1 complex. *Cell* **103**, 569–581 (2000).
66. Ludwig, T., Griffiths, G. & Hoflack, B. Distribution of newly synthesized lysosomal enzymes in the endocytic pathway of normal rat kidney cells. *J. Cell Biol.* **115**, 1561–1572 (1991).
67. Press, B., Feng, Y., Hoflack, B. & Wandingerness, A. Mutant rab7 causes the accumulation of cathepsin D and cation-independent mannose 6-phosphate receptor in an early endocytic compartment. *J. Cell Biol.* **140**, 1075–1089 (1998).
68. Bucci, C., Thomsen, P., Nicoziani, P., McCarthy, J. & van Deurs, B. Rab7: a key to lysosome biogenesis. *Mol. Biol. Cell* **11**, 467–480 (2000).
69. Shiba, Y., Takatsu, H., Shin, H. W. & Nakayama, K. γ-adaptin interacts directly with rabaptin-5 through its ear domain. *J. Biochem.* **131**, 327–336 (2002).
70. Stenmark, H., Vitale, G., Ullrich, O. & Zerial, M. Rabaptin-5 is a direct effector of the small GTPase Rab5 in endocytic membrane fusion. *Cell* **83**, 423–432 (1995).
71. Bock, J. B., Klumperman, J., Davanger, S. & Scheller, R. H. Syntaxin 6 functions in *trans*-Golgi network vesicle trafficking. *Mol. Biol. Cell* **8**, 1261–1271 (1997).
72. Prekeris, R., Klumperman, J., Chen, Y. A. & Scheller, R. H. Syntaxin 13 mediates cycling of plasma membrane proteins via tubulovesicular recycling endosomes. *J. Cell Biol.* **143**, 957–971 (1998).
73. Steegmaier, M., Klumperman, J., Foletti, D. L., Yoo, J. S. & Scheller, R. H. Vesicle-associated membrane protein 4 is implicated in *trans*-Golgi network vesicle trafficking. *Mol. Biol. Cell* **10**, 1957–1972 (1999).
74. Klumperman, J., Kuliawat, R., Griffith, J. M., Geuze, H. J. & Arvan, P. Mannose 6-phosphate receptors are sorted from immature secretory granules via adaptor protein AP-1, clathrin, and syntaxin 6-positive vesicles. *J. Cell Biol.* **141**, 359–371 (1998).
75. Peden, A. A., Park, G. Y. & Scheller, R. H. The di-leucine motif of vesicle-associated membrane protein 4 is required for its localization and AP-1 binding. *J. Biol. Chem.* **276**, 49183–49187 (2001).
76. Simonsen, A., Gaullier, J. M., D'Arrigo, A. & Stenmark, H. The Rab5 effector EEA1 interacts directly with syntaxin-6. *J. Biol. Chem*. **274**, 28857–28860 (1999).
77. Storrie, B. & Desjardins, M. The biogenesis of lysosomes: is it a kiss and run, continuous fusion and fission process? *Bioessays* **18**, 895–903 (1996).
78. Schweizer, A., Kornfeld, S. & Rohrer, J. Proper sorting of the cation-dependent mannose 6-phosphate receptor in endosomes depends on a pair of aromatic amino acids in its cytoplasmic tail. *Proc. Natl Acad. Sci. USA* **94**, 14471–14476 (1997).
This work established the importance of a di-aromatic sequence on the cytoplasmic tail of the CD-MPR in preventing it from entering lysosomes.
79. Diaz, E. & Pfeffer, S. R. TIP47: a cargo selection device for mannose 6-phosphate receptor trafficking. *Cell* **93**, 433–443 (1998).
80. Orsel, J. G., Sincock, P. M., Krise, J. P. & Pfeffer, S. R. Recognition of the 300-kDa mannose 6-phosphate receptor cytoplasmic domain by 47-kDa tail-interacting protein. *Proc. Natl Acad. Sci. USA* **97**, 9047–9051(2000).
81. Carroll, K. S. *et al*. Role of Rab9 GTPase in facilitating receptor recruitment by TIP47. *Science* **292**, 1373–1376 (2001).

82. Barbero, P., Bittova, L. & Pfeffer, S. R. Visualization of Rab9-mediated vesicle transport from endosomes to the *trans*-Golgi in living cells. *J. Cell Biol.* **156**, 511–518 (2002).
83. Riederer, M. A., Soldati, T., Shapiro, A. D., Lin, J. & Pfeffer, S. R. Lysosome biogenesis requires Rab9 function and receptor recycling from endosomes to the *trans*-Golgi network. *J. Cell Biol*. **125**, 573–582 (1994).
References 79–83 provide evidence that TIP47/Rab9 mediates sorting of the MPRs at the late endosome and has a role in retrograde trafficking to the Golgi.
84. Meyer, C. *et al*. μ1A-adaptin-deficient mice: lethality, loss of AP-1 binding and rerouting of mannose 6-phosphate receptors. *EMBO J.* **19**, 2193–2203 (2000).
85. Meyer, C., Eskelinen, E. L., Guruprasad, M. R., von Figura, K. & Schu, P. μ1A deficiency induces a profound increase in MPR300/IGF-II receptor internalization rate. *J. Cell Sci.* **114**, 4469–4476 (2001).
References 84 and 85 were the first studies to implicate AP1 in retrograde trafficking of MPRs from endosomes to the *trans*-Golgi network.
86. Wan, L. *et al*. PACS-1 defines a novel gene family of cytosolic sorting proteins required for *trans*-Golgi network localization. *Cell* **94**, 205–216 (1998).
87. Crump, C. M. *et al*. PACS-1 binding to adaptors is required for acidic cluster motif-mediated protein traffic. *EMBO J.* **20**, 2191–2201 (2001).
This reference cites the first evidence in support of PACS-1/AP1-mediated retrograde transport of MPRs from early endosomes to the *trans*-Golgi network.
88. Tikkanen, R. *et al*. The dileucine motif within the tail of the MPR46 is required for sorting of the receptor in endosomes. *Traffic* **1**, 631–640 (2000).
89. Wasiak, S. *et al*. Enthoprotin: a novel clathrin-associated protein identified through subcellular proteomics. *J. Cell Biol.* **158**, 855–862 (2002).
90. Jadot, M., Canfield, W. M., Gregory, W. & Kornfeld, S. Characterization of the signal for rapid internalization of the bovine mannose 6-phosphate/insulin-like growth factor-II receptor. *J. Biol. Chem.* **267**, 11069–11077 (1992).
91. Johnson, K. F., Chan, W. & Kornfeld, S. Cation-dependent mannose 6-phosphate receptor contains two internalization signals in its cytoplasmic domain. *Proc. Natl Acad. Sci. USA* **87**, 10010–10014 (1990).
92. Denzer, K., Weber, B., Hille-Rehfeld, A., von Figura, K. & Pohlmann, R. Identification of three internalization sequences in the cytoplasmic tail of the 46 kDa mannose 6-phosphate receptor. *Biochem. J.* **326**, 497–505 (1997).
93. Storch, S. & Braulke, T. Multiple C-terminal motifs of the 46-kDa mannose 6-phosphate receptor tail contribute to efficient binding of medium chains of AP-2 and AP-3. *J. Biol. Chem.* **276**, 4298–4303 (2001).
94. Ludwig, T. *et al*. Mouse mutants lacking the type 2 IGF receptor (IGF2R) are rescued from perinatal lethality in IGF2 and IGF1R null backgrounds. *Dev. Biol.* **177**, 517–535 (1996).
95. Wang, Z. Q., Fung, M. R., Barlow, D. P. & Wagner, E. F. Regulation of embryonic growth and lysosomal targeting by the imprinted *IGF2/MPR* gene. *Nature* **372**, 464–467 (1994).
96. Lau, M. M. *et al*. Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. *Genes Dev.* **8**, 2953–2963 (1994).
97. Ikezu, T., Okamoto, T., Giambarella, U., Yokota, T. & Nishimoto, I. *In vivo* coupling of insulin-like growth factor II/mannose 6-phosphate receptor to heteromeric G proteins. Distinct roles of cytoplasmic domains and signal sequestration by the receptor. *J. Biol. Chem.* **270**, 29224–29228 (1995).
98. Zhang, Q. *et al*. Insulin-like growth factor II signaling through the insulin-like growth factor II/mannose 6-phosphate receptor promotes exocytosis in insulin-secreting cells. *Proc. Natl Acad. Sci. USA* **94**, 6232–6237 (1997).
99. McKinnon, T., Chakraborty, C., Gleeson, L. M., Chidiac, P. & Lala, P. K. Stimulation of human extravillous trophoblast migration by IGF-II is mediated by IGF type 2 receptor involving inhibitory G protein(s) and phosphorylation of MAPK. *J. Clin. Endocrinol. Metab.* **86**, 3665–3674 (2001).
100. Groskopf, J. C., Syu, L. J., Saltiel, A. R. & Linzer, D. I. Proliferin induces endothelial cell chemotaxis through a G protein-coupled, mitogen-activated protein kinase-dependent pathway. *Endocrinology* **138**, 2835–2840 (1997).
101. Minniti, C. P. *et al*. The insulin-like growth factor II (IGF-II)/mannose 6-phosphate receptor mediates IGF-II-induced motility in human rhabdomyosarcoma cells. *J. Biol. Chem*. **267**, 9000–9004 (1992).
102. Tsuruta, J. K., Eddy, E. M. & O'Brien, D. A. Insulin-like growth factor-II/cation-independent mannose 6-phosphate receptor mediates paracrine interactions during spermatogonial development. *Biol. Reprod.* **63**, 1006–1013 (2000).
103. Ikushima, H. *et al*. Internalization of CD26 by mannose 6-phosphate/insulin-like growth factor II receptor contributes to T cell activation. *Proc. Natl Acad. Sci. USA* **97**, 8439–8444 (2000).
104. Nishimoto, I. The IGF-II receptor system: a G protein-linked mechanism. *Mol. Reprod. Dev.* **35**, 398–406 (1993).
105. Frasca, F. *et al*. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol. Cell. Biol*. **19**, 3278–3288 (1999).
106. Korner, C., Nurnberg, B., Uhde, M. & Braulke, T. Mannose 6-phosphate/insulin-like growth factor II receptor fails to interact with G-proteins. Analysis of mutant cytoplasmic receptor domains. *J. Biol. Chem*. **270**, 287–295 (1995).
107. Ikushima, H. *et al*. Soluble CD26/dipeptidyl peptidase IV enhances transendothelial migration via its interaction with mannose 6-phosphate/insulin-like growth factor II receptor. *Cell. Immunol*. **215**, 106–110 (2002).
108. Purchio, A. F. *et al*. Identification of mannose 6-phosphate in two asparagine-linked sugar chains of recombinant transforming growth factor-β1 precursor. *J. Biol. Chem.* **263**, 14211–14215 (1988).
109. Crawford, S. E. *et al*. Thrombospondin-1 is a major activator of TGF-β1 *in vivo*. *Cell* **93**, 1159–1170 (1998).
110. Dennis, P. A. & Rifkin, D. B. Cellular activation of latent transforming growth factor β requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. *Proc. Natl Acad. Sci. USA* **88**, 580–584 (1991).
This work showed for the first time that the CI-MPR plays a significant role in activation of the latent form of TGF-β1.
111. Nunes, I., Shapiro, R. L. & Rifkin, D. B. Characterization of latent TGF-β activation by murine peritoneal macrophages. *J. Immunol.* **155**, 1450–1459 (1995).
112. Godar, S. *et al*. M6P/IGFII-receptor complexes urokinase receptor and plasminogen for activation of transforming growth factor-β1. *Eur. J. Immunol.* **29**, 1004–1013 (1999).
113. Ghahary, A. Tredget, E. E. & Shen, Q. Insulin-like growth factor-II/mannose 6 phosphate receptors facilitate the matrix effects of latent transforming growth factor-β1 released from genetically modified keratinocytes in a fibroblast/keratinocyte co-culture system. *J. Cell Physiol.* **180**, 61–70 (1999).
114. Chen, A., Davis, B. H., Sitrin, M. D., Brasitus, T. A. & Bissonnette, M. Transforming growth factor-β1 signaling contributes to Caco cell growth inhibition induced by 1,25(OH)2D3. *Am. J. Physiol. Gastrointest. Liver Physiol.* **283**, G864–G874 (2002).
115. Leksa, V. *et al*. The N-terminus of mannose 6-phosphate/insulin-like growth factor 2 receptor in regulation of fibrinolysis and cell migration. *J. Biol. Chem*. **277**, 40575–40582 (2002).
116. Nykjaer, A. *et al*. Mannose 6-phosphate /insulin-like growth factor–II receptor targets the urokinase receptor to lysosomes via a novel binding interaction. *J. Cell Biol.* **141**, 815 –828 (1998).
117. Kang, J. X., Bell, J., Beard, R. L. & Chandraratna, R. A. Mannose 6-phosphate/insulin-like growth factor II receptor mediates the growth-inhibitory effects of retinoids. *Cell Growth Differ.* **10**, 591–600 (1999).
118. Zaina, S. & Squire, S. The soluble type 2 insulin-like growth factor (IGF-II) receptor reduces organ size by IGF-II-mediated and IGF-II-independent mechanisms. *J. Biol. Chem.* **273**, 28610–28616 (1998).
119. O'Gorman, D. B., Weiss, J., Hettiaratchi, A., Firth, S. M. & Scott, C. D. Insulin-like growth factor-II/mannose 6-phosphate receptor overexpression reduces growth of choriocarcinoma cells *in vitro* and *in vivo*. *Endocrinology* **143**, 4287–4294 (2002).
120. DeSouza, A. T., Hankins, G. R., Washington, M. K., Orton, T. C. & Jirtle, R. L. *M6P/IGF2R* gene is mutated in human hepatocellular carcinomas with loss of heterozygosity. *Nature Genet.* **11**, 447–449 (1995).
The first evidence that CI-MPR is mutated in human cancers.
121. Yamada, T., DeSouza, A. T., Finkelstein, S. & Jirtle, R. L. Loss of the gene encoding mannose 6-phosphate/insulin-like growth factor II receptor is an early event in liver carcinogenesis. *Proc. Natl Acad. Sci. USA* **94**, 10351–10355 (1997).
122. Oka, Y. *et al*. M6P/IGF2R tumor suppressor gene mutated in hepatocellular carcinomas in Japan. *Hepatology* **35**, 1153–1163 (2002).
123. Hankins, G. R. *et al*. M6P/IGF2 receptor: a candidate breast tumor suppressor gene. *Oncogene* **12**, 2003–2009 (1996).
124. Chappell, S. A., Walsh, T., Walker, R. A. & Shaw, J. A. Loss of heterozygosity at the mannose 6-phosphate insulin-like growth factor 2 receptor gene correlates with poor differentiation in early breast carcinomas. *Br. J. Cancer* **76**, 1558–1561 (1997).
125. Kong, F. M., Anscher, M. S., Washington, M. K., Killian, J. K. & Jirtle, R. L. M6P/IGF2R is mutated in squamous cell carcinoma of the lung. *Oncogene* **19**, 1572–1578 (2000).
126. Rey, J. M., Theillet, C., Brouillet, J. P. & Rochefort, H. Stable amino-acid sequence of the mannose-6-phosphate/insulin-like growth-factor-II receptor in ovarian carcinomas with loss of heterozygosity and in breast-cancer cell lines. *Int. J. Cancer* **85**, 466–473 (2000).
127. Leboulleux, S., Gaston, V., Boulle, N., LeBouc, Y. & Gicquel, C. Loss of heterozygosity at the mannose 6-phosphate/insulin-like growth factor receptor locus: a frequent but late event in adrenocortical tumorigenesis. *Eur. J. Endocrinol.* **144**, 163–168 (2001).
128. Gemma, A. *et al*. Mutation analysis of the gene encoding the human mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) in human cell lines resistant to growth inhibition by transforming growth factor β_1 (TGF-β_1). *Lung Cancer* **30**, 91–98 (2000).
129. Byrd, J. C., Devi, G. R., DeSouza, A. T., Jirtle, R. L. & MacDonald, R. G. Disruption of ligand binding to the insulin-like growth factor II/mannose 6-phosphate receptor by cancer-associated missense mutations. *J. Biol. Chem.* **274**, 24408–24416 (1999).
130. Devi, G. R., DeSouza, A. T., Byrd, J. C., Jirtle, R. L. & MacDonald, R. G. Altered ligand binding by insulin-like growth factor II/mannose 6-phosphate receptors bearing missense mutations in human cancers. *Cancer Res.* **59**, 4314–4319 (1999).
References 128–130 show that cancer-associated mutations in the CI-MPR impair receptor function.
131. Collins, B. M., McCoy, A. J., Kent, H. M., Evans, P. R. & Owen, D. J. Molecular architecture and functional model of the endocytic AP 2 complex. *Cell* **109**, 523–535 (2002).
132. Ricotta, D., Conner, S. D., Schmid, S. L., von Figura, K. & Honing, S. Phosphorylation of the AP2 μ-subunit by AAK1 mediates high affinity binding to membrane protein sorting signals. *J. Cell Biol.* **156**, 791–795 (2002).
133. DaCosta, S. A., Schumaker, L. M. & Ellis, M. J. Mannose 6-phosphate/insulin-like growth factor 2 receptor, a bona fide tumor suppressor gene or just a promising candidate? *J. Mammary Gland Biol. Neoplasia* **5**, 85–94 (2000).
134. Motyka, B. *et al*. Mannose 6-phosphate/insulin-like growth factor II receptor is a death receptor for granzyme B during cytotoxic T cell-induced apoptosis. *Cell* **103**, 491–500 (2000).

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Online links

DATABASES

The following terms in this article are linked online to:

Swiss-Prot: http://www.expasy.ch/

CD-MPR | CD26 | CI-MPR | EEA1 | GGA1 | GGA2 | GGA3 | granzyme B | IGF-binding protein 5 | IGF-I | IGF-II | plasminogen | rabaptin5 | syntaxin 6 | syntaxin 13 | TGF-β1 | TIP47 | uPAR | VAMP-4

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