J. Membrane Biol. 103, 7–16 (1988)

The Journal of
Membrane Biology

Topical Review

Mannose 6-Phosphate Receptors and Their Role in Targeting Proteins to Lysosomes

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Introduction

Proteins destined for the cell surface, secretory storage granules, and lysosomes share a common route of transport through the secretory pathway (*see* Pfeffer & Rothman, 1987, for review). Cleavable signal peptides present on all of these proteins direct them to the endoplasmic reticulum (ER), and they are translocated across the membrane of this organelle during their synthesis. Within the ER, they receive high-mannose, asparagine-linked oligosaccharides, disulfide bridges are formed, proteins fold, and, where applicable, they assemble into their correct oligomeric structures. This collection of proteins with different destinations is then transported together in membrane-bound vesicles to the Golgi complex. Here, their oligosaccharide chains are trimmed and remodeled, and it is within the Golgi complex that proteins are somehow sorted to their appropriate final destinations.

In the first (or *cis*) compartment of the Golgi complex, lysosomal hydrolases are selectively recognized by the enzyme, N-acetylglucosamine phosphotransferase, which adds P-GlcNAc to mannose residues present on their asparagine-linked oligosaccharides (von Figura & Hasilik, 1986). GlcNAc is then removed, and in this manner, lysosomal hydrolases acquire a mannose 6-phosphate moiety. Lysosomal enzymes then proceed through the remainder of the Golgi complex (from the *cis* to medial to *trans* Golgi), and it is not until they reach the last compartment of the Golgi complex, the *trans* Golgi network (Griffiths & Simons, 1986), that they are segregated into distinct transport vesicles and away from other cell surface and secreted proteins.

Key Words mannose 6-phosphate receptors · lysosomal enzymes · IGF-II receptor · protein sorting

Receptors specific for phosphomannosyl-containing oligosaccharides are present in the Golgi complex, and facilitate the segregation of lysosomal enzymes from proteins with other destinations within the secretory pathway. Two distinct mannose 6-phosphate (man6P) receptors have been identified. One of these requires the presence of divalent cations for ligand binding in vitro; the other does not. This review will summarize our current picture of man6P receptors and their role in lysosomal enzyme sorting. Several excellent, detailed reviews focussing on these and related topics have also recently appeared (Sly & Fischer, 1982; von Figura & Hasilik, 1986; Kornfeld, 1986, 1987; Sahagian, 1987) and are recommended to the reader.

The Cation-Independent Mannose 6-Phosphate Receptor

Kaplan and co-workers (1977*a*,*b*) were the first to demonstrate that a hexose-phosphate, specifically man6P, is essential for the high affinity and saturable endocytosis of lysosomal hydrolases by cultured cells (*see* Sly & Fischer, 1982, for review). This observation enabled Sahagian, Distler and Jourdian (1981) to use affinity chromatography to purify a phosphomannosyl receptor of apparent molecular weight 215,000 from bovine liver. Immobilized mammalian (Sahagian et al., 1981) and *Dictyostelium discoideum* (Fischer, Creek & Sly, 1982; Varki & Kornfeld, 1983) lysosomal enzymes, in addition to immobilized core and pentamannosyl 6-phosphate fragments from the phosphomannan of the yeast, *Hansenula holstii* (Steiner & Rome, 1982; Brown & Farquhar, 1984) have since been employed to purify this cation-independent receptor from a variety of sources.

The cation-independent man6P receptor is a

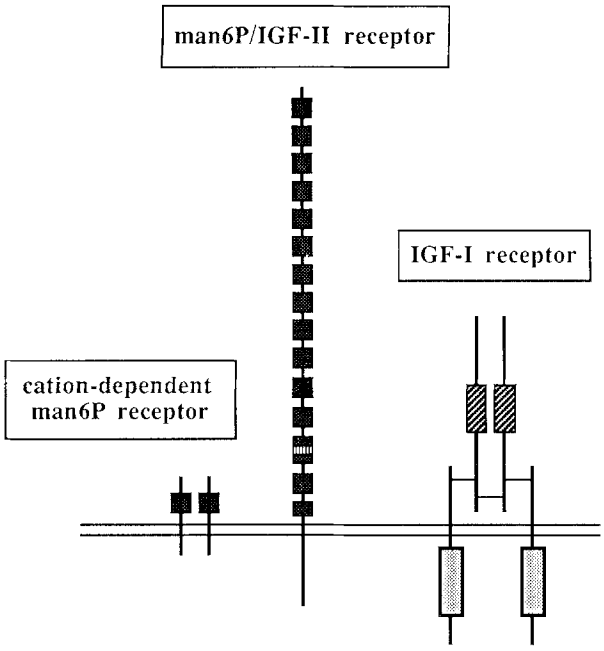


Fig. 1. Schematic comparison of mannose 6-phosphate receptor structures. Characteristic repeat units in the man6P receptor extracellular portions are represented as boxes; the vertical stripes represent sequences homologous to fibronectin (adapted from Dahms et al., 1987, Lobel et al., 1987, and Morgan et al., 1987). The structure of the IGF-I receptor is shown for comparison (Ebina et al., 1986). A distinct cysteine-rich region in the IGF-I receptor is depicted by diagonal stripes; the intracellular tyrosine kinase domain is screened

transmembrane glycoprotein (von Figura, Gieselmann & Hasilik, 1985; Sahagian & Steer, 1985) that contains numerous intrachain disulfide bridges, since its mobility is significantly altered in nonreducing gels (Sahagian & Neufeld, 1983). After insertion into the ER membrane, the receptor requires as long as 2 to 3 hr to properly fold and be exported to the Golgi complex (Sahagian & Neufeld, 1983). This is significantly longer than the time required for a number of other membrane proteins to be exported from the ER (Lodish, 1988), and may be due to the complexity of intrachain disulfide bond formation. In the Golgi complex, the receptor acquires complex-type oligosaccharide structures and then undergoes a repeated cycle of serine residue phosphorylation and dephosphorylation, the significance of which is not yet clear (Sahagian & Neufeld, 1983).

The complete primary sequence of the cation-independent man6P receptor has recently been deduced from cDNA clones encoding this protein (Lobel et al., 1987; Morgan et al., 1987; Lobel, Dahms & Kornfeld, 1988; Oshima et al., 1988). The mature protein has a polypeptide backbone that is about 270,000 daltons in mass. Since approximately 20,000–30,000 daltons of the protein's mass is likely to be contributed by carbohydrate, the actual molecular weight of the cation-independent receptor is probably close to 300,000.

A single, hydrophobic stretch divides the human 300 kilodalton receptor into extracellular and intracellular domains of 2,265 and 164 amino-acid residues, respectively (Fig. 1; Morgan et al., 1987; Oshima et al., 1988). Analogous domains in the bovine receptor are 2269 and 163 residues in length (Lobel et al., 1988). Nineteen potential asparagine-linked oligosaccharide addition sites are present in the extracellular domain of these proteins.

The entire extracellular portion of the 300 kD receptor is comprised of 15 sequence repeat units, which are each about 145 residues in length, and are characterized by a conserved spacing of cysteine residues flanked by conserved hydrophobic residues. Each repeat unit contains a highly conserved, 13 amino-acid stretch that matches the consensus sequence:

cys (thr/glu) tyr *x* phe (glu) trp *x* thr *x* (ala/val) ala cys.[1]

While the structural and functional significance of these repeats is not yet clear, it is important to note that the cysteine repeat unit is distinct from that seen in other receptors possessing cysteine-rich extracellular domains (Yarden & Ullrich, 1988).

The 15 repeat units are interrupted only once by a 43 amino-acid insertion which bears 53% sequence identity with the type II region of fibronectin. In fibronectin, this disulfide-bonded region contributes to a collagen binding domain (Kornbliht et al., 1985). Tandem repeats of such sequences have also been detected in the blood clotting factor XII (McMullen & Fujikawa, 1985) and in a bovine seminal fluid protein (Esch et al., 1983). Why the 300 kD man6P receptor contains sequences homologous to fibronectin is not yet known.

Figure 2 presents a comparison of the cytoplasmic tail sequences of bovine, rat, and human cation-independent man6P receptors, which are 163, 167, and 164 amino acids in length, respectively. The cytoplasmic domain is rather acidic and is the most divergent region between the bovine, rat, and human proteins. Within this domain, one can identify highly conserved regions (Fig. 2, boxed), which are surrounded by significantly more divergent sequences that display only 38% identity. The cytoplasmic domain of this receptor is almost twice the

[1] Residues shown in parentheses show a lower percentage match to the consensus; *x* denotes positions that are not conserved.

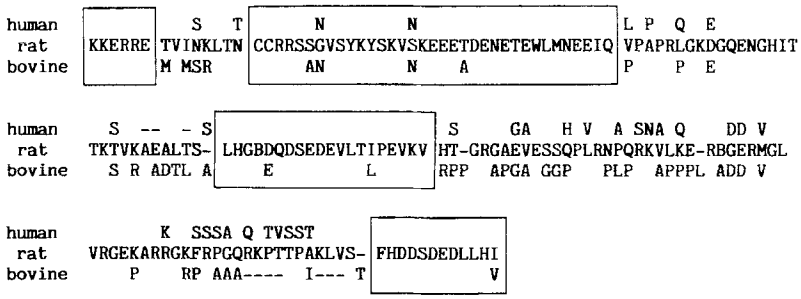


Fig. 2. Comparison of the cytoplasmic domain sequences of rat, human, and bovine cation-independent man6P receptors. The full rat sequence is shown (MacDonald et al., 1988); only differences are shown for the human (upper lines, Morgan et al., 1987; MacDonald et al., 1988) and bovine (lower lines, Lobel et al., 1987) sequences. Homologous regions are boxed

size of the corresponding domain of the cation-dependent man6P receptor (below) and that of other transport receptors such as those for LDL, transferrin, and asialoglycoproteins.

The Cation-Dependent Mannose 6-Phosphate Receptor

A number of cultured cell lines have been shown to lack the 300 kD man6P receptor. These include macrophage ($P388D_1$ and J774), fibroblast (L cells), myeloma (MOPC315 cells), and hepatoma (Morris hepatoma 7777) cells (Gabel, Goldberg & Kornfeld, 1983; Stein et al., 1987*b*). Despite the absence of the 300 kD receptor, these cell lines are still able to direct lysosomal hydrolases accurately to lysosomes. In their efforts to elucidate the basis for lysosomal enzyme targeting in such cells, Hoflack and Kornfeld (1985*a*) identified the existence of a second, smaller man6P receptor, which appears to require divalent cations for binding to phosphomannosyl residue-containing lysosomal enzymes in vitro. These workers purified the cation-dependent man6P receptor to homogeneity from bovine liver and mouse $P388D_1$ cells, and showed that it is a glycosylated protein of about 46,000 daltons (Hoflack & Kornfeld, 1985*b*) and is likely to exist as a dimer (Hoflack & Kornfeld, 1985*b*; Stein et al., 1987*b*). The deglycosylated protein has a molecular weight of approximately 28,000, thus a large portion of the receptor's mass is contributed by carbohydrate (Hoflack & Kornfeld, 1985*b*).

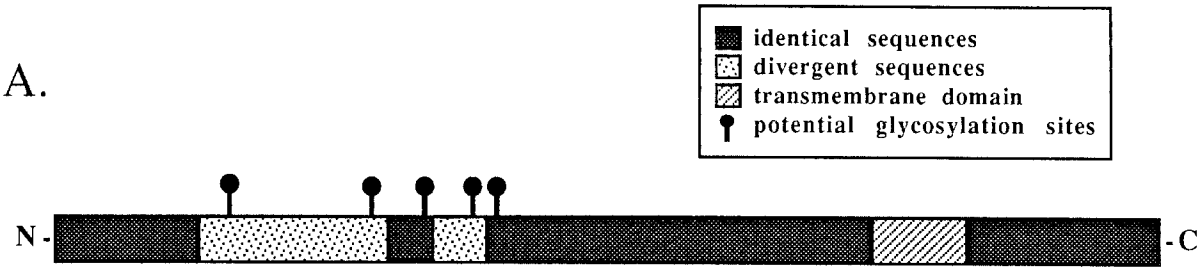
The primary sequences of both bovine and human 46 kD receptors have recently been deduced from cDNA clones encoding these proteins (Dahms et al., 1987; Pohlmann et al., 1987). The mature proteins are each comprised of 257 amino acids, and span the membrane once, exposing 67 amino acids to the cytoplasm (Fig. 1). Five sites for potential asparagine-linked oligosaccharide addition are found clustered near the amino terminus, and biochemical studies indicate that at least four of these sites are modified (Hoflack & Kornfeld, 1985*b*; Dahms et al., 1987; Stein et al., 1987*b*). A very striking feature of the cation-dependent man6P receptor sequence is that the extracellular portion possesses a single copy of the same characteristic, ~145 residue long, cysteine-rich structural repeat that appears 15 times in the 300 kD receptor.

Direct comparison of the bovine and human protein sequences reveals that they are 96% identical, differing at only 11 amino-acid positions (Fig. 3). Surprisingly, all of these differences are clustered near the amino terminus, which enables classification of receptor structural domains within the extracellular portion of the 46 kD receptor. The first 28 amino acids of the mature proteins are identical. Then, a variable amino terminal region is followed by a cluster of closely spaced, oligosaccharide addition sites. This glycosylated region is followed by 75 amino acids that are absolutely conserved between bovine and human receptors. As noted by Dahms et al. (1987), it is this portion of the receptor that contains the most highly conserved, 13 amino-acid sequence that is present in each of the sequence repeats in the cation-independent man6P receptor. It is quite reasonable to propose that at least part of the ligand binding domain will be localized to this membrane-proximal, conserved region.

Considering the importance of the phosphate group in phosphomannosyl recognition by the 46 kD receptor (Hoflack, Fujimoto & Kornfeld, 1987), it would not be at all surprising if arginine and/or lysine groups were utilized for key ionic interactions with the phosphate moiety within the ligand binding pocket. In the case of the enzyme phosphofructokinase, three arginine residues are used to form salt bridges with the phosphate group of fructose 6-phosphate (Evans, Farrants & Hudson, 1981). Indeed, Stein and co-workers (1987*a*) have recently shown that arginine residues contribute to the ligand binding of the 46 kD receptor. Modification of these residues with 1,2-cyclohexanedione abolished ligand binding; however, binding was much less severely affected if man6P was added prior to arginine modification to protect the ligand binding site.

Comparison of the crystal structures of a num-

A.



B.

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val arg glu ser trp gln thr glu glu lys thr cys asp leu val gly glu lys gly lys

                                                                  *
glu ser glu lys glu leu ala leu leu lys arg leu thr pro leu phe asn lys ser phe
                              val             lys

glu ser thr val gly gln ser pro asp met tyr ser tyr val phe arg val cys arg glu
                        gly ser     thr     ile     ile

          *                                         *
ala gly asn his ser ser gly ala gly leu val gln ile asn lys ser asn gly lys glu
            thr

                  *                         *
thr val val gly arg phe asn glu thr gln ile phe asn gly ser asn trp ile met leu
                    leu             his

ile tyr lys gly gly asp glu tyr asp asn his cys gly arg glu gln arg arg ala val
                                                lys

val met ile ser cys asn arg his thr leu ala asp asn phe asn pro val ser glu glu

arg gly lys val gln asp cys phe tyr leu phe glu met asp ser ser leu ala cys ser

pro glu ile ser his leu ser val gly ser ile leu leu val thr leu ala ser leu val
                                                          phe

ala val tyr ile ile gly gly phe leu tyr gln arg leu val val gly ala lys gly met
            val val

glu gln phe pro his leu ala phe trp gln asp leu gly asn leu val ala asp gly cys

asp phe val cys arg ser lys pro arg asn val pro ala ala tyr arg gly val gly asp

asp gln leu gly glu glu ser glu glu arg asp asp his leu leu pro met
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Fig. 3. Comparison of bovine and human cation-dependent man6P receptor sequences. (*A*) A schematic diagram showing conserved and divergent regions. (*B*) The complete bovine sequence is shown (Dahms et al., 1987); only human sequence differences are shown below (Pohlmann et al., 1987). Asterisks denote sites for asparagine-linked glycosylation; the putative transmembrane domain is boxed

ber of carbohydrate-binding proteins has revealed the importance of hydrogen bonds in ligand binding (*see* Quiocho, 1986, for review). The individual planar side chains of amino acids such as arginine, aspartic acid, glutamic acid, and asparagine are often utilized to form multiple hydrogen bonds with specific ligand sugars. The conserved membrane-proximal domain of the 46 kD receptors is especially rich in this category of amino acids, which could be utilized both for the formation of salt bridges (arg & lys) and in hydrogen bonding interactions. The availability of cDNA clones encoding

both receptors will enable site-specific mutagenesis experiments to pinpoint the specific residues that are essential for ligand binding.

Unlike the cytoplasmic tail of the 300 kD receptor, the 67 amino-acid cytoplasmic domains of the bovine and human 46 kD receptors are absolutely conserved. They are comparable in size to the analogous domain of the LDL receptor (67 *vs.* 50 residues). The cytoplasmic domain may function in homo-oligomeric interactions that might be required for accurate intracellular targeting between cellular compartments, as has been suggested for the LDL receptor (Davis et al., 1987; van Driel et al., 1987).

Role of Man6P Receptors in Lysosomal Enzyme Targeting

That the phosphomannosyl recognition system is important for lysosomal enzyme targeting is demonstrated rather dramatically in I-cell disease (*see* von Figura & Hasilik, 1986, for review). Patients with I-cell disease lack the enzyme UDP-GlcNAc phosphotransferase (Hasilik, Waheed & von Figura, 1981; Reitman, Varki & Kornfeld, 1981), thus their lysosomal hydrolases fail to acquire man6P. In the fibroblasts of these patients, lysosomes contain only barely detectable levels of a number of soluble hydrolytic enzymes, and large amounts of these proteins are secreted.

Both the cation-dependent and cation-independent man6P receptors have been directly implicated in the recognition and targeting of man6P-containing proteins to lysosomes. As will be described below, man6P receptors are located primarily within the cell; however, they all appear briefly at the cell surface (and are then re-internalized) over a period of a few hours (von Figura, Gieselman & Hasilik, 1984; Sahagian, 1984). When cells are incubated with antibodies that block the ligand binding sites of either receptor, the efficiency of lysosomal enzyme targeting is decreased (von Figura et al., 1984; Gartung et al., 1985; Nolan et al., 1987; Stein et al., 1987*c*). Under these conditions, a large proportion of man6P receptors come in contact with the antibodies, and lysosomal enzymes, unable to bind to man6P receptors, are secreted from the cells. These experiments demonstrate that *both* the 300 and 46 kD receptors play a key role in lysosomal enzyme targeting.

In the case of the LDL receptor, naturally occurring mutations have greatly facilitated analyses of receptor function (Goldstein et al., 1985). No patients have yet been discovered with a defect in a man6P receptor (*see* Kornfeld, 1986, for review). The introduction into cultured cells of in vitro-generated receptor mutants may add to our understanding of the cellular functions of the two man6P receptors.

Intracellular Transport of Mannose 6-Phosphate Receptors

A number of laboratories have undertaken immunocytochemical and biochemical studies to determine the cellular distribution of the man6P receptors. First, it is clear that man6P receptors are not present in mature lysosomes, by either biochemical (Sahagian & Neufeld, 1983; von Figura et al., 1984; Stein et al., 1987*b*) or morphological (Willingham et al., 1981; Geuze et al., 1984; Brown, Goodhouse & Farquhar, 1986) criteria. The receptors are found in the Golgi complex and in endosomes; a small amount (less than 5–10%) are present on cell surfaces.

Where do man6P receptors first encounter newly synthesized lysosomal enzymes? Man6P receptors present on the surface and within the cell are in rapid equilibrium, since over 90% contact extracellularly administered antibodies within a few hours (von Figura et al., 1984; Sahagian, 1984; Gartung et al., 1985; Nolan et al., 1987; Pfeffer, 1987). Duncan and Kornfeld (1988) took advantage of this fact to monitor the transport of man6P receptors, labeled at the cell surface, back to the Golgi complex. These investigators found that both cation-dependent and independent man6P receptors recycled back to the site of sialyltransferase (*trans* Golgi network and *trans* Golgi) with a half-time of about 3 hr. However, transport to a more distal Golgi compartment (the medial Golgi) was barely detectable. These experiments strongly suggest that man6P receptors bind to lysosomal hydrolases in the *trans* Golgi network and sort them from secreted and plasma membrane proteins within this compartment (Duncan & Kornfeld, 1988).

After binding lysosomal hydrolases in the *trans* Golgi network, man6P receptor-ligand complexes are collected into clathrin-coated pits which bud off to form clathrin-coated vesicles (*see* von Figura and Hasilik (1986) for review). These transport vesicles deliver the receptors to an acidic endosomal compartment, where lysosomal hydrolases are released (Gonzalez-Noriega et al., 1980; Sahagian, 1984; Brown et al., 1986). Griffiths and co-workers (1988) have recently completed a detailed electron-microscopic study of the localization of the 300 kD man6P receptor in NRK cells, using immunogold labeling procedures. These workers have identified an "intermediate compartment" which they propose is

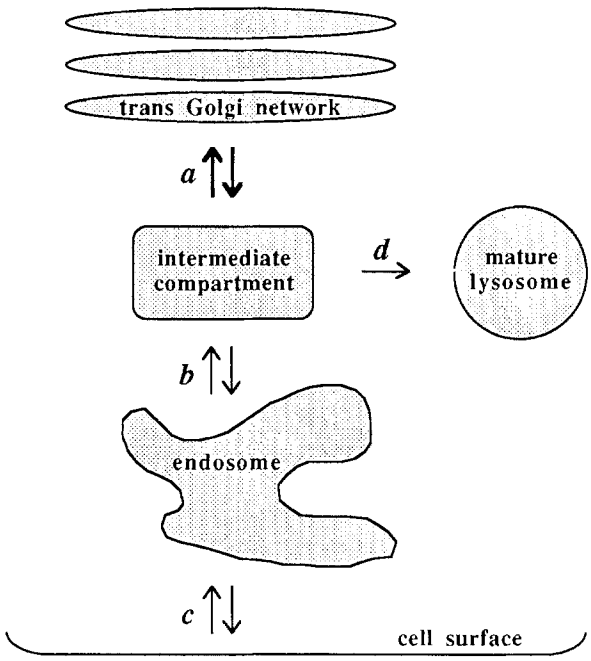

Fig. 4. Model for the intracellular transport of proteins en route to lysosomes. Man6P receptors bind to newly synthesized lysosomal enzymes in the *trans* Golgi network and carry them to an intermediate compartment (step *a*). Ligands are released from receptors in this compartment due to the low intraorganellar pH, and man6P receptors recycle back to the *trans* Golgi. A small number of man6P receptors are also present at the cell surface and can internalize extracellular lysosomal enzymes and deliver them to the intermediate compartment by passage through endosomes (steps *c* and *b*). Ligands delivered to the intermediate compartment (but not man6P receptors) end up in mature lysosomes (step *d*)

the acidic delivery site for newly synthesized, receptor-bound lysosomal hydrolases en route to lysosomes. The intermediate compartment is located near the *trans* Golgi network, yet it is a distinct compartment, because it does not contain a viral glycoprotein that accumulates in the *trans* Golgi network at 20°C. Unlike the *trans* Golgi network, the intermediate compartment is highly enriched in both 300 kD man6P receptors and lysosomal membrane glycoproteins, and furthermore, the intermediate compartment appears to be significantly more acidic than the *trans* Golgi network. At temperatures above 20°C, the intermediate compartment receives endocytosed proteins. Taken together, these findings suggest that this newly identified compartment represents a prelysosome or "late-endosome" structure.

The intermediate compartment shares an important feature with endosomes: unlike lysosomes, sorting takes place within this organelle, since man6P receptors are presumably retrieved from this compartment and returned to the Golgi complex. Because of this distinction, it may be most accurate to categorize the intermediate compartment as a type of "late endosome."

Figure 4 summarizes our current model for the vesicular transport pathways taken by man6P receptors. Man6P receptors shuttle primarily between the *trans* Golgi network and the so-called, intermediate compartment (step *a*, Fig. 4). Newly synthesized lysosomal enzymes bind to receptors in the *trans* Golgi network and are delivered to the intermediate compartment; receptors then recycle back to the *trans* Golgi network for another round of transport. Man6P receptors can also bind to extracellular lysosomal enzymes at the cell surface and deliver them to the intermediate compartment (steps *c* & *b*). Lysosomal enzymes delivered to the intermediate compartment later appear in mature lysosomes (step *d*). This step may involve maturation of an intermediate compartment into a lysosome (Helenius et al., 1983).

Since all man6P receptors appear briefly at the cell surface, transport is likely to be possible from the intermediate compartment back to the cell surface (steps *b* & *c*). It is important to note, however, that transport from the intermediate compartment to endosomes (step *b*, Fig. 4) has not yet been demonstrated. The possibility that some man6P receptors are mis-sorted and arrive at the cell surface directly from the *trans* Golgi network seems unlikely, but cannot yet be ruled out.

Comparison of the Two Man6P Receptors

It is not yet clear why cells have two types of man6P receptors. One could have imagined that the two proteins carried out different functions, bound different classes of lysosomal enzymes, or were present in different cell types or cellular locations. At present, none of these possibilities appears to be correct.[2] The 46 and 300 kD receptors share a broad tissue distribution, and while the two receptors are present in roughly equimolar amounts in bovine liver (Hoflack & Kornfeld, 1985*b*), the 46 kD receptor is the predominant phosphomannosyl binding protein in homogenates of bovine testes (Distler & Jourdian, 1987; Distler, Patel & Jourdian, 1987). As described earlier, a few cell lines lack the 300 kD

[2] The precise intracellular distribution of the 46 kD receptor, as determined by electron microscopy, has not yet been reported. However, biochemical experiments by Stein et al. (1987*b*) and by Duncan and Kornfeld (1988) indicate that its distribution is likely to be very similar to that of the 300 kD receptor.

man6P receptor. No cell line has yet been identified that lacks the 46 kD receptor, and most cell types appear to contain both.

The 46 and 300 kD man6P receptors are specific for oligosaccharides bearing phosphomannosyl groups in monoester linkage, and bind with highest affinity to oligosaccharides containing two such moieties (Creek & Sly, 1982; Fischer et al., 1982; Varki & Kornfeld, 1983; Hoflack et al., 1987). Both proteins release their ligands at pH values below 5.5, a characteristic feature of receptors that deliver their cargo to acidic intracellular compartments (Sahagian et al., 1981; Hoflack & Kornfeld, 1985*a*,*b*). While similar to the 300 kD receptor in its binding properties, the 46 kD receptor can be distinguished from the 300 kD receptor in that it does not bind to methylphosphomannosyl groups present on *Dictyostelium discoideum* lysosomal enzymes (Hoflack & Kornfeld, 1985*a*), and it displays a distinct pH profile for binding to lysosomal enzymes in vitro (Hoflack & Kornfeld, 1987). However, Hoflack et al. (1987) were unable to detect any differences between the two receptors with regard to their ability to bind to any particular subset of lysosomal enzymes.

Despite similar binding properties, the primary sequences of the 46 and 300 kD receptors do not display a high degree of sequence identity; the only apparent common feature is the presence of the ~145 amino-acid long, cysteine-rich repeat units. However, the repeat units appear not to represent individual man6P binding domains, since the 300 kD receptor and a 46 kD receptor dimer each bind 2 moles of man6P (Kornfeld, 1987), far less than the number of repeat units (15) present in the 300 kD receptor. Variable numbers of another type of cysteine-rich repeat occur in other cell surface receptors, including those for LDL, EGF, and insulin (Pfeffer & Ullrich, 1986). Since multiple copies of this cysteine-rich repeat are found in cell surface receptors with very diverse functions, they appear to form an important structural element in the vicinity of ligand binding domains, *without* defining binding specificity. Yet, a puzzle remains: why do two proteins with the same apparent function have such disparate structures?

The 300 kD Man6P Receptor is Identical to the IGF-II Receptor

The deduced amino-acid sequence of the insulin-like growth factor type II receptor has recently been obtained from full-length cDNA clones (Morgan et al., 1987). Quite unexpectedly, the human sequence was found to be over 80% identical with the sequence of the bovine cation-independent man6P receptor (Lobel et al., 1987, 1988), and more recently, over 99% identical with the sequence of the human cation-independent man6P receptor (Oshima et al., 1988). Does one of these cloned receptors have a mistaken identity? Apparently not. First, in each case, the isolated cDNA clones contained extensive, known peptide sequences derived from independently purified, IGF-II and 300 kD man6P receptor preparations. Morgan and co-workers (1987) expressed their cloned IGF-II receptor cDNA in oocytes and observed the appearance of IGF-II binding sites on the surfaces of these cells. Expression of the human man6P receptor cDNA generated a protein that could mediate β-glucuronidase endocytosis (Oshima et al., 1988). Thus, these essentially identical cDNA's encode a protein capable of binding both IGF-II and lysosomal enzymes.

Several additional lines of evidence demonstrate that the IGF-II receptor and the 300 kD man6P receptor are in fact identical proteins. MacDonald et al. (1988) have shown that in crude extracts, a majority of IGF-II binding activity is retained on a column of pentamannosyl 6-phosphate and can be specifically eluted with man6P, demonstrating that both binding activities are present in a single polypeptide. Furthermore, several groups have demonstrated that the affinity of the 300 kD receptor for IGF-II appears to increase twofold in the presence of man6P (Roth et al., 1987; MacDonald et al., 1988; but *see also* Tong et al., 1988). It is important to note that the man6P/IGF-II receptor binds both ligands with high affinity, in the nanomolar range.

These results demonstrate that a single receptor protein has two distinct ligand binding sites that interact cooperatively. The 300 kD man6P/IGF-II receptor would be expected to contain two distinct binding sites, since the binding of lysosomal enzymes to this receptor absolutely requires the presence of man6P groups, and IGF-II is not even glycosylated. In hindsight, a review of the characteristics of the protein studied either as the "IGF-II receptor" or as the "cation-independent, man6P receptor" reveals numerous similarities, including similar apparent molecular weights, high disulfide bridge content, broad tissue distribution, and a primarily intracellular localization.

The presence of distinct binding sites for both IGF-II and man6P-containing proteins may in part explain why the 300 kD receptor is larger than the 46 kD man6P receptor, which does not bind IGF-II (Tong, Tollefsen & Kornfeld, 1988). The possibility remains that the 300 kD receptor binds yet another ligand (or ligands), in addition to IGF-II and proteins that contain man6P.

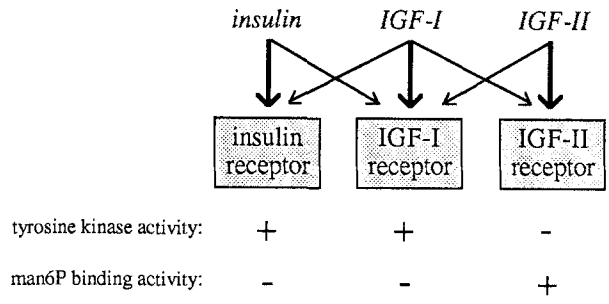

Fig. 5. Properties of insulin and insulin-like growth factor receptors. The ligands (shown at the top) interact with the designated receptors (*see* text)

Significance of a Multifunctional Receptor

In bacterial chemotaxis, a single receptor senses the concentrations of both aspartate and maltose (bound to the maltose binding protein), and can trigger a chemotactic response to either or both of these components, independently and additively (Mowbray & Koshland, 1987). In this case, bacterial cells use a single receptor to couple two ligands to the same chemotactic signalling machinery. Might there also be a connection between the physiological consequences of binding both IGF-II and lysosomal enzymes to a single receptor? Alternatively, do the two binding sites reflect independent functions of this receptor protein? Although the 300 kD receptor appears to play a key role in targeting enzymes to lysosomes (described above), the significance of this molecule as an IGF-II binding protein is not yet fully established.

The first issue that must be considered is whether the man6P/IGF-II receptor transduces a growth factor signal. If the receptor transmits a signal across the plasma membrane, it would be all the more difficult to reconcile why the same receptor protein is used by the cell to target proteins to lysosomes and to generate an intracellular signal. The importance of this issue becomes clear when one considers the complexities of ligand interaction with insulin and insulin-like growth factor receptors.

Insulin and the related growth factors, IGF-I and IGF-II, are structurally homologous growth factors that bind to specific, cognate cell surface receptors (*see* Czech, 1982; Rechler & Nissley, 1985, for review). These growth factors also bind to heterologous members of this receptor family with somewhat lower affinities in vitro (Fig. 5). Thus, IGF-I can interact with both insulin and IGF-II receptors, and insulin and IGF-II can also bind to the IGF-I receptor.[3] Insulin and IGF-I receptors are highly homologous proteins that possess an intrinsic tyrosine kinase activity that is essential for signal transduction (Ebina et al., 1985; Ullrich et al., 1985, 1986). In contrast, the primary sequence of the IGF-II receptor is completely unrelated to that of the insulin and IGF-I receptors (Morgan et al., 1987), and the IGF-II receptor lacks tyrosine kinase activity in vitro (Corvera et al., 1986). The absence of sequence homology between IGF-I and IGF-II receptors suggests that the IGF-II binding sites present on both of these proteins either interact with different portions of the IGF-II molecule, or alternatively consist of similarly folded protein domains.

Since IGF-II can activate the tyrosine kinase activity of the IGF-I receptor, it has been extremely difficult to dissociate the physiological effects of IGF-II that are mediated by its *own* receptor from those triggered by the IGF-I receptor. Thus, while several reports have suggested that the IGF-II receptor does indeed transduce a signal (Hari et al., 1987; Nishimoto et al., 1987*a*,*b*; Tally, Li & Hall, 1987), others have concluded that it does not (Mottola & Czech, 1984; Krett, Heaton & Gelehrter, 1987).

The simplest explanation for the presence of two ligand binding sites on the 300 kD receptor is that this receptor is merely used to clear IGF-II from the circulation. For unknown reasons, IGF-II is present at rather high levels (300–600 ng/ml, or ~60 nM) in human serum. By internalizing IGF-II and releasing it within endosomes, the 300 kD receptor would target IGF-II to lysosomes for degradation. In this model, a different protein would be used to mediate an IGF-II signal: either the IGF-I receptor, or another, yet to be identified, IGF-II binding protein.

If the man6P/IGF-II receptor does indeed transduce a growth factor signal, the consequences of intracellular stimulation of the receptor, in cell types such as liver that express both the receptor *and* its cognate growth factor, must be considered. However, the cytoplasmic tail of a signal-transducing receptor might be expected to have been more highly conserved (Fig. 2).

It has been known for several years that treatment of cells with insulin causes a redistribution of intracellular IGF-II(/man6P) receptors to the cell surface (Oka, Mattola & Czech, 1984; Wardzala et al., 1984). Perhaps the 300 kD receptor is used to sense the circulating concentration of IGF-II, and in

[3] IGF-II can apparently also interact with the insulin receptor, but only at very high concentrations in vitro.

some way regulate its level of expression in liver. In this regard, IGF-II is expressed at high levels during early development, and its expression is coordinated with that of a serum IGF-II binding protein as well as the man6P/IGF-II receptor (A. Ullrich, *personal communication*). Careful analysis of this insulin-stimulated, redistribution process may offer clues to some of the physiologically important roles played by this receptor.

Future Perspectives

The analysis of man6P receptors has uncovered a very satisfying pathway for lysosomal enzyme sorting, yet it is clear that man6P receptors accomplish only part of this sorting task. An indication of the existence of a man6P-*independent* sorting pathway has also come from the analysis of cells from patients with I-cell disease. As described earlier, such cells are unable to construct man6P-containing oligosaccharides. Paradoxically, while I-cell fibroblasts are deficient in several lysosomal enzymes (*see above*), intracellular levels of lysosomal enzymes are near normal in liver, spleen, kidney and brain (Owada & Neufeld, 1982; Waheed et al., 1982). The basis for this targeting is presently unknown.

Recent characterization of a number of *membrane-associated* lysosomal proteins, including a family of lysosomal membrane glycoproteins of unknown function (Chen et al., 1985; Lewis et al., 1985; Barriocanal et al., 1986; Lippincott-Schwartz & Fambrough, 1986), has revealed that none of these proteins acquires man6P groups on their oligosaccharide side chains. Therefore, some other mechanism must account for their accurate targeting to lysosomes. It is tempting to speculate that the same sorting machinery that is responsible for the targeting of man6P receptors (which themselves lack man6P residues) to the intermediate compartment may also segregate lysosomal membrane proteins and direct them to this destination (Green et al., 1987). Whatever signals are involved in this sorting must be unique to this class of membrane proteins.

The existence of man6P-independent sorting pathways makes it very clear that man6P receptors are themselves not responsible for sorting. Instead, they represent an efficiency mechanism by which the cell can couple perhaps as many as 50 different lysosomal enzymes to a single sorting machinery. A major challenge for the future will be to elucidate the molecular components of this sorting machinery, and to establish how the cell segregates man6P receptors and lysosomal membrane glycoproteins away from proteins bound for other cellular destinations.

Research in the author's laboratory is supported by grants from the National Institutes of Health (DK37332), the March of Dimes Birth Defects Foundation (5-635), and the Weingart Foundation for Biomedical Research.

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Received 8 January 1988; revised 4 March 1988