0031-3998/95/3806-0835$03.00/0
PEDIATRIC RESEARCH

Vol. 38, No. 6, 1995
Printed in U.S.A.

Receptor Cell Biology: Receptor-Mediated Endocytosis

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ABSTRACT

Receptor-mediated endocytosis (RME) provides one major pathway for the trafficking of extracellular molecules into the cell. This involves the binding of a ligand to a specific cell surface receptor, clustering of the ligand-receptor complexes in coated pits, invagination and pinching off of the coated pits to form coated vesicles, and delivery of coated vesicles to discrete membrane-limited cytoplasmic sorting organelles, endosomes. Within these endosomes, ligands and receptors are each targeted to their appropriate cellular destination (*e.g.*, lysosome, cytoplasm, opposite cell surface). The cell and molecular biologic basis for such a tightly regulated process is now beginning to be understood and is reviewed herein. (***Pediatr Res* 38: 835–843, 1995**)

Abbreviations
RME, receptor-mediated endocytosis
ASGP, asialoglycoprotein
EGF, epidermal growth factor
AP, adaptins
CURL, compartment of uncoupling receptor and ligand
Man-6-P, mannose 6-phosphate
t-PA, tissue-type plasminogen activator
u-PA, urokinase plasminogen activator plasminogen activator inhibitor type-1 (PAI-1)

The fundamental processes responsible for growth and development in man are exceedingly complex. The availability of cell nutrients as well as growth factors and hormones is essential for normal tissue differentiation. One of the major mechanisms responsible for delivery of these nutrient molecules/growth factors to cells is via the process termed RME. This system is responsible for the bulk of macromolecular transport into the developing egg, across the placenta, across the neonatal gut, and into the cells of every organ during human development. In addition to the receptors mediating endocytosis and macromolecular transport, cellular receptors govern transmembrane signaling (*e.g.* for hormones and neurotransmitters) as well as intracellular signals (*e.g.* for steroid and thyroid hormones).

Receptor biology has a long and glorious history. Curare, used for centuries by the Indians of the Amazon basin as an arrow poison, was brought to England by Sir Walter Raleigh in the 16th century. Claude Bernard began a systematic investigation of its action in 1850. But it was J. N. Langley, examining the antagonistic effect of curare on nicotine stimulation of skeletal muscle nearly a century ago, who concluded: "Since neither curare nor nicotine, even in large doses, prevents direct stimulation of muscle from causing contraction, it is obvious that the muscle substance which combines with nicotine or curare is not identical with the substance which contracts. It is convenient to have a term for the specially excitable constituent, and I have called it the receptive substance. It receives the stimulus, and by transmitting it, causes contraction" (1). These two principles—the recognition capacity for specific ligands and the subsequent ability of the ligand-receptor complex to initiate a biologic response—form the basis of our current understanding of receptor biology. However, the recent explosive growth in the basic biologic understanding of receptor biology in the past few years precludes an exhaustive review of all these systems. Thus, instead of a superficial overview of a multitude of systems, I have concentrated on a focused review of one such process, RME.

RME is a specific cellular biologic process by which various macromolecules bind to cell surface receptors and are subsequently internalized and trafficked within the cell. Ligands internalized via RME represent a wide variety of macromolecules with varying physiologic activities including: nutrient provision [*e.g.* LDL (2, 3), transferrin (4)]; modified molecules from the circulation [*e.g.* ASGP (5, 6), plasminogen activator-inhibitor complexes (7)]; hormones [*e.g.* insulin, EGF (8, 9)]; and some lysosomal enzymes (10). In addition, certain viruses and toxins (11, 12) use this pathway to gain entrance to the cell. This review provides a general description of the cellular mechanisms and physiology of RME and intracellular trafficking of ligand-receptor complexes, the specific receptors com-

Manuscript dedicated to Professor H.K.A. Visser in honor of his retirement.

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monly found on liver cells, the signals responsible for endocytosis and intracellular targeting and the regulation of the RME pathway.

MECHANISMS OF RME

Most cellular functions require an intracellular environment isolated from that of the extracellular space. The cell membrane provides such a physical barrier to control macromolecule movement. RME provides one major pathway for the trafficking of extracellular molecules into the cell and involves the binding of a ligand to a specific cell surface receptor, clustering of the ligand-receptor complexes in coated pits, invagination and pinching off of the coated pits to form coated vesicles, and delivery of coated vesicles to discrete membrane-limited cytoplasmic organelles (*e.g.* endosome/CURL). A model of RME is depicted in Figure 1. Each step is discussed in detail below.

Receptor movement to coated pits. Entry of ligand-receptor complexes into cells occurs through specialized membrane areas called coated pits. Clustering of receptor proteins within coated pits is the initial step of RME. This process may be either spontaneous or triggered by ligands. Some receptors, such as those for LDL (13) and transferrin (14), appear to be clustered in coated pits independent of prior ligand binding. Other receptors, including those for EGF (15), insulin (16), and ASGP (17), are evenly distributed over the plasma membrane. Several studies suggest that the rate of receptor diffusion on the cell surface plasma membrane is sufficient to explain the movement into coated pits (18, 19). Other evidence suggests that membrane lipids flow toward coated pit areas (20), suggesting that all membrane proteins may be carried passively to coated pits (21, 22). However, only those receptor proteins mediating endocytosis are "trapped" via specific signals in the cytoplasmic tail which interact with structural components of the coated pit (see below).

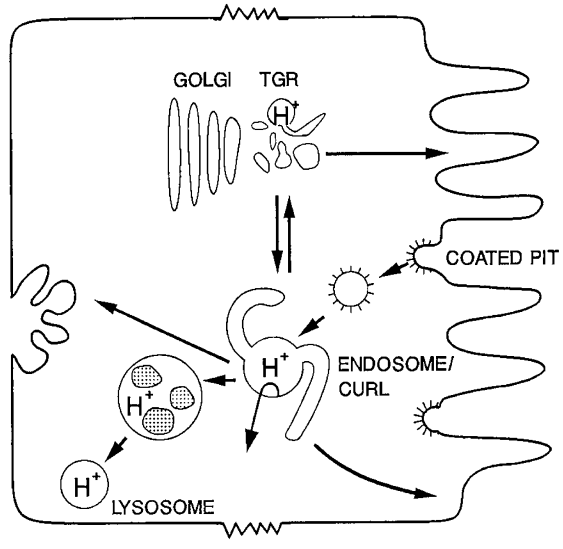

Figure 1. RME. Ligand molecules bind to specific cell surface receptors at the plasma membrane. After clustering in coated pits and internalization via coated vesicles, the ligand-receptor complexes are delivered to the endosomal sorting compartments in the peripheral cytoplasm. Within these tubulovesicular organelles (*i.e.* endosome/CURL) acidification of the internal contents via a specific proton pump promotes appropriate sorting of various ligands and receptors. Each ligand and each receptor is specifically targeted to its appropriate destination with fidelity. Some are trafficked to the lysosome via multivesicular endosomes. Others are recycled back to the plasma membrane. Still others are directed across the cell in a process termed *transcytosis* or are targeted into the cytoplasm directly.

Coated pits. The coated pit is the cellular machinery for ligand-receptor internalization. The name derives from the characteristic fuzzy, basket-like coat on the cytoplasmic surface of the cell membrane as observed with electron microscopy (23). In most cells coated pits occupy approximately 2% of the plasma surface area (24, 25). Coated pits continuously invaginate and transform into coated vesicles at a rate of approximately 3×10^3 $min^{-1}cell^{-1}$ (26). This continuous transformation requires rapid replenishment of cell surface coated pits probably via coated pit recycling (27). Although the number of coated pits at the cell surface may be regulated under certain conditions (28), in general this step of RME appears to be constitutive.

The coat of both coated pits and coated vesicles consists of a highly ordered array of specific macromolecules organized into a polygonal lattice (29). Studies on these components revealed that the basic structural unit of the coat is composed of three molecules of clathrin heavy chain (180 kD) and three molecules of clathrin light chain (33–36 kD). The three light chains overlap with the three heavy chains to give rise to a three-legged structure termed "triskelion" (30, 31). In addition to clathrin heavy and light chains, coated vesicles also contain a second major class of proteins which have been termed adaptor proteins or AP (32–35). Two heterotetrameric, structurally related classes of AP are present in most cells. AP1 consists of 100–110 kD, τ, and β' AP and two smaller subunits of 47 and 49 kD. AP2 consists of 100–110 kD α and β AP and two smaller subunits of about 50 and 17 kD (35, 36). AP appear as barrel-shaped molecules with two appendages attached via a flexible stalk (37). AP appear to function in clathrin assembly. The positioning of AP between the clathrin lattice and the vesicle membrane suggests that AP function in mediating clathrin binding to vesicle membranes. Recent studies suggest that the β or β' subunits mediate the binding to clathrin (38), whereas the other subunits may function in binding receptor molecules (29).

The morphology of coated pits has been observed in replicas of cells that are quick-frozen, freeze-fractured, and deep-etched (39). The coats observed under these conditions appeared to form by piecemeal assembly, not by coalescence of large patches. Some typical lattices seen on the inner surfaces of chick fibroblasts are shown in Figure 2, which is arranged to illustrate a progressive increase in the extent of microcage nucleation around the edges of lattice. Although the precise molecular mechanisms of coated pit assembly are still poorly understood, *in vitro* reconstitution systems for the assembly of cytoplasmically derived proteins into coated pits have been developed (40). These systems which directly measure coated pit assembly, invagination, and budding should allow for detailed morphologic, biochemical, and kinetic dissection of the processes responsible for RME.

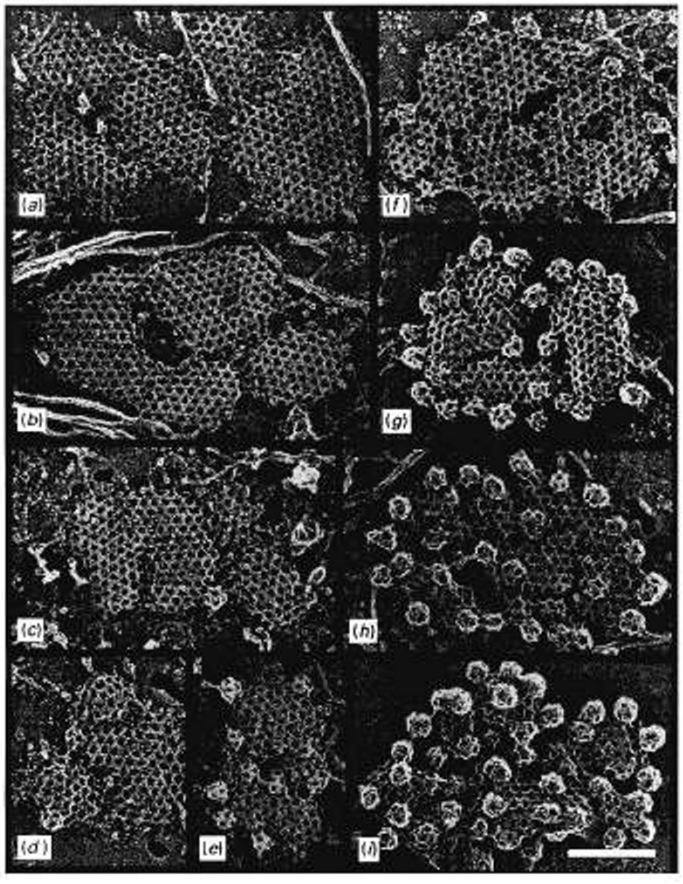

Figure 2. Gallery of typical replica images of clathrin lattices seen on the inner surfaces of chick fibroblasts exposed to a variety of acidifying conditions. *Bar* = 0.2 μm. [Adapted from Steer and Heuser (133).]

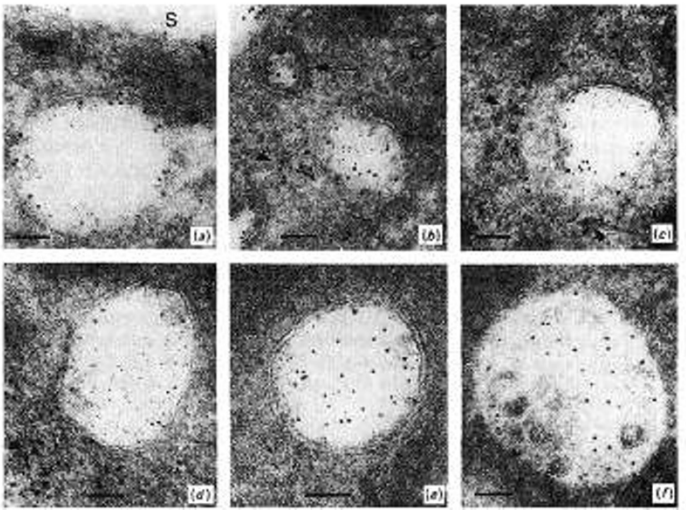

Figure 3. Receptor and ligand are segregated within endosome/CURL. Immunoelectron micrographs of ultrathin cryosections from rat liver labeled with two sizes of colloidal gold. Livers were continuously infused with asialofetuin 1 h before fixation. (*a*) A vesicle just beneath the sinusoidal (*S*) plasma membrane with ligand (5 nm of gold) bound to the ASGP receptor (8 nm gold). (*b*) A coated pit (*arrow*) demonstrating receptor (8 nm of gold) and ligand (5 nm of gold). The slightly tangential view of CURL shows a heterogeneous distribution of receptor in the vesicular portion and abundant receptor in associated tubules (*arrowhead*). (*c*) The CURL profile shows peripheral ligand (5 nm of gold) and heterogeneous labeling of receptor (8 nm of gold). Intense receptor labeling is present over the tubules adjacent to the vesicular portion of CURL (*arrowheads*). (*d*) Free ligand (5 nm of gold) can be seen in the lumen of the vesicle portion of CURL. Receptor labeling (8 nm of gold) is scarce and heterogeneous there but intense over the connecting tubules (*arrowhead*). (*e*) CURL profile in which receptor (5 nm gold) labeling is located predominantly at the pole where a tubule is connected (*arrow*). The majority of ligand (8 nm of gold) is present free in the vesicle lumen. (*f*) A multivesicular body containing unbound ligand (8 nm of gold) in its matrix. No labeling of receptor (5 nm of gold) is seen. *Bars* represent 0.1 μm (16). [Adaped from Gruze *et al.* (17).]

FATES OF LIGANDS AND RECEPTORS

Sorting in the endosome/CURL. The generic name "endosomes" describes the entire family of related organelles which constitute intermediates in the pathway of RME (41). Specific subsets of endosomes have been defined by virtue of selected structural and/or physiologic characteristics. For example, CURL refers specifically to those endosomal sorting organelles in which ligand-receptor dissociation occurs (17, 42) (Fig. 3). Irrespective of their nomenclature, all endocytotic sorting organelles have a rather characteristic structure/function relationship. To date these organelles have been identified by virtue of being intermediates along the endocytotic routes (*i.e.* no specific molecular markers exist) (43). Thus, most studies have characterized their morphology on the basis of ligand physiology. Studies with electron opaque tracers attached to ligands destined for receptor-mediated uptake (44–46) have demonstrated that the bulk of endocytotic structures are concentrated at the peripheral cytoplasm.

Endosomes are tubulovesicular structures of various sizes and shapes (17, 42, 47). Upon endocytosis a ligand initially encounters endosomes with relatively small vesicular volumes. Thereafter, the vesicular volume as well as extent of tubular membrane increases (42). About three-fourths of the total membrane is within the tubular portions, whereas about three-fourths of the volume is within the vesicular portion. Some investigators have suggested an anastomosing network (48–50). These tubulovesicular organelles are found within virtually every cell (except perhaps the mature erythrocyte) (17, 42, 51). The vesicular portions have diameters of 0.1–0.5 μm, whereas the diameter of the tubular portions is approximately 0.05 μm. Quantitative immunoelectron microscopy has demonstrated striking differences in the distribution of ligands and receptors within the tubulovesicular structures. At least three distinct domains can be defined at present: tubular membrane, vesicular membrane, and vesicular contents. Geuze *et al.* (17) have demonstrated the striking localization of dissociated ligand within the vesicular contents (Fig. 4). Receptor (an integral membrane protein) was not present within this domain but was confined to the tubular and vesicular membrane. The relative receptor labeling in the tubular *versus* vesicular membranes correlates closely with the diameter of the vesicle (42); with large vesiculotubular structures demonstrating virtually all receptors confined to the tubular portions of the organelle.

Quantitatively, it is difficult to precisely define all endosomal organelles within the cell. Nonetheless, stereologic observations suggest approximately 200–300 endocytotic vesicles per cell with a total fractional volume of approximately 2.5% (52). In contrast, lysosomes were approximately four to five times more abundant but also accounted for only 2.5% cellular volume. Although only a few of cellular volume endosomes

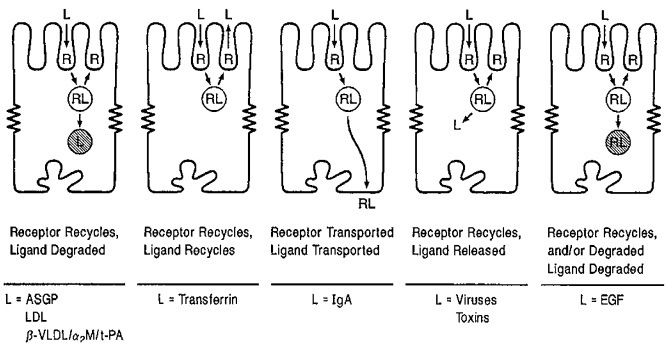

Figure 4. Intracellular sorting pathways of RME. The initial steps (including receptor clustering in coated pits, internalization of ligand-receptor complexes to form coated vesicles, and fusion of vesicles to form endosomes) are common to all the pathways. However, after entry into acidic endosomes, ligands and receptors can be sorted and targeted differently for different systems. The IgA receptor is transported together with its ligand across the hepatocyte in the form of a secretory component (see text). *L*, ligand; *R*, receptor; lysosomes are *shadowed*.

may comprise more than 25% of total cellular membrane (53). In most cells, endocytosed ligand enters the peripheral endosomal tubulovesicular organelles and later is found in larger vesicular structures which contain internal membrane (*i.e.*, multivesicular bodies/multivesicular endosomes) before delivery to classical lysosomes. This multivesicular endosome appears to be devoid of tubular extensions and is approximately as abundant as the tubulovesicular endosomes.

To define the molecular structure and function of endosomes, several laboratories have attempted to isolate these organelles using a variety of approaches. A major difficulty encountered by all relates to the vast heterogeneity in size, shape, and contents, as well as delicate nature of the structures. Four general approaches have been taken. Each relies on a procedure for physical separation after cell disruption and homogenization: native density separation, native charge separation, ligand-induced density or charge separation, and immunoselection (47, 54–56).

Despite the relative inhomogeneity of endosome preparations, substantial progress has been achieved in defining the properties responsible for endosomal function. In large part these derive from *in vitro* reconstitution systems and *in situ* physiologic regulatory studies. Central to the elucidation of endosome function is the requirement that ligands, receptors, and endosomal constituents be properly sorted within the cell. Thus, segregation of ligands targeted to the lysosome must be separated from ligands transported across the cell or ligands recycled in and out of the cell. One central physiologic event common to all of these sorting events is the fusion of membrane-limited compartments with one another. Thus, the initial coated vesicle delivers its contents to endosomes, which also receive ligands (*e.g.* lysosomal enzymes) from the secretory route via the Golgi (57). Several laboratories have developed cell-free endocytotic systems (58–62). Common features of all of these systems of vesicular traffic include the requirement for ATP and cytosolic factors (58, 63). Acidification of endocytotic vesicles is not essential for fusion. Most recently, two additional cytoplasmic regulatory components have been defined for these *in vitro* systems (protein kinase-mediated phosphorylation (64) and small GTPases (65).

Acidification of endosomes was first described in intact cells using fluorescent labels on endocytosed ligands (66). Similar approaches were used in cell-free systems (67, 68). Endosome acidification is the result of activation of an ATPase distinct from that in gastric cells, or mitochondria (69). The pH in endosomes decreases to about 5.3 (in contrast to lysosomes which may reach 4.0) (66, 70). The consequences of this endosomal acidification are profound. Many of the ligand-receptor complexes dissociate within this acidic environment, thus providing a mechanism for the sorting of ligand from receptor. However, the pH sensitivities of various ligand-receptor complexes is distinct. This feature may provide a mechanism for the selected sorting of various ligands/receptors from one another. Indeed, acidification may also induce conformational changes in receptor structure (71). This may be sufficient to promote segregation of receptors within the lateral membranes of the endosome and may potentially underlie membrane protein sorting within microdomains of the tubulovesicular structures (42, 72).

Regulation of endosomal pH. The regulation of the endosomal pH is one of the most important factors for appropriate routing of molecules along the endocytotic pathways. By following the fate of a ligand destined for delivery to lysosomes and by making observations at various times and in various cellular locations, these studies suggest that early endosomes maintain a pH of 6 which falls to 5–5.5 in large endosomes over 15–20 min and ultimately to 4.6–5 in lysosomes (73, 74). Although these studies are remarkably consistent from cell type to cell type for ligands destined for direct delivery to lysosomes, there are alternative pathways within most cells. The best studied of these is the recycling pathway of transferrin and the transferrin receptor (75, 76). This ligand is spared lysosomal delivery but passes through the *trans*-Golgi reticulum (see below). Following the fate of transferrin fluorescence, Yamashiro *et al.* (73) have demonstrated the movement from early endosomes (pH 6) to less acidic (pH 6.4) recycling organelles in the *trans*-Golgi region.

Thus, various intracellular organelles along the endocytotic route maintain pH values from below 7 to as low as 4.5. Endosomes, lysosomes, and coated vesicles all have ATP-dependent proton pumping activity (77–79). The vacuolar proton pumps in endosomes, lysosomes, and coated vesicles are distinct from several other well known proton pumps including those present in mitochondria, gastric membranes, and yeast. A major consequence of endosomal acidification is the selective sorting of molecules along the routes of intracellular transport (Fig. 4).

Role of Golgi and the trans-Golgi reticulum. Endocytosis involves the uptake of macromolecules from outside of the cell with delivery to intracellular sorting sites and often the lysosomes as discussed above. Secretion, on the other hand, was thought until recently to be separate and distinct from endocytosis and involves vesicular trafficking of molecules from the rough endoplasmic reticulum to the Golgi and ultimately to the plasma membrane. Recent biochemical, morphologic, and kinetic studies have clearly defined a linkage between these two

pathways. Molecules of the secretory pathway make their way to the endocytotic pathway and *vice versa* (80, 81).

INTERNALIZED LIGANDS AND THEIR RECEPTORS

Since the initial studies of the RME, many internalized ligands and their receptors have been identified and characterized. Most of the early studies focused on kinetic analyses of ligand binding and internalization. Characterization of the molecular nature of the receptor proteins has been greatly enhanced by the recent molecular cloning of receptor genes. The properties of several representative endocytic receptors and their ligands are discussed below (see Fig. 5).

LDL and its receptor. One of the best characterized systems of RME is the uptake of LDL (3). LDL is a lipoprotein complex whose principal function is to serve as a carrier of water-insoluble cholesterol and cholesterol esters in the serum. A single molecule of apolipoprotein B mediates receptor binding. Intracellular release of cholesterol after internalization and degradation of LDL has several important metabolic consequences for the cell including: *1*) reduction of the biosynthesis of cholesterol by the cell; *2*) activation of acyltransferase which is responsible for esterifying cholesterol (*i.e.* cholesterol storage); and *3*) suppression of the synthesis of new LDL receptors which prevents overaccumulation of cholesterol from exogenous sources (82).

The LDL receptor is a 160-kD glycoprotein with abundant *O*-linked carbohydrate (83). Molecular cloning and sequencing of the receptor cDNA (84), together with biochemical characterization (85), revealed that the mature receptor is divided into five distinct domains which include, starting from the NH_2 terminus, a ligand binding domain, an EGF-precursor-like domain, an *O*-linked sugar domain, a membrane-spanning domain, and a cytoplasmic tail. The receptor is oriented in the plasma membrane with its NH_2 terminus facing the extracellular space. Human mutants with various molecular defects in the LDL receptor have defined many of the molecular features important to RME (see below).

ASGP and their receptor. The hepatic ASGP receptor is an integral membrane glycoprotein which exists as two proteins, the product of the two genes (6). The major species (46 kD) (86, 87) is approximately 4-fold more abundant than the minor species (50 kD) (88). The sequences share approximately 50% homology. The polypeptides are oriented with NH_2 terminus in the cytoplasm and span the plasma membrane once. The receptor moves from the rough endoplasmic reticulum to the Golgi and thereafter to the *trans*-Golgi reticulum and plasma membrane (86, 87). The major and minor species assemble together into a functional multisubunit receptor somewhere along the secretory route. Therein both subunits assemble with a stoichiometry of ~4:1 (46 kD:50 kD) which appears to be essential for proper ligand binding and intracellular routing (89, 90).

The ASGP receptor recognizes ligands with terminal galactose/galactosamine residues. Upon ligand binding, ligand and receptor are rapidly internalized and are delivered to endosome/CURL where ligand/receptor sorting occurs via acidification (90). Liberated ligand is predominantly (~80%) directed to lysosomes for rapid catabolism, whereas approximately 20% of ligand is recycled back to the cell surface both free and receptor-bound (91). The ASGP receptor within endosome/CURL is spared lysosomal degradation and is efficiently recycled back to the sinusoidal cell surface to participate in subsequent rounds of endocytosis (17). An entire receptor cycle occurs as rapidly as 8 min, and a single ASGP receptor may recycle 250–300 times in its lifetime (90).

Transferrin and its receptor. Iron transport is a two-step process which involves the binding of iron to the serum glycoprotein, transferrin, and their subsequent delivery to cells via the transferrin receptor. Each transferrin molecule has two binding sites for ferric ions. After entering the cell together with transferrin, iron molecules dissociate from the transferrin, are transported to the cytoplasm, and are used or stored (76). Apotransferrin recycles back to the cell surface in association with the receptor and is subsequently released to the medium where it can be reutilized as an iron carrier (75). The transferrin receptor is a dimeric glycoprotein (180 kD) composed of two identical disulfide-linked 90-kD subunits (92, 93).

Insulin and EGF and their receptors. Two of the better characterized growth factors are insulin and EGF. The insulin receptor is a tetrameric complex composed of two α subunits (130 kD) (which bind insulin) and two β subunits (90 kD) (94). Upon insulin binding to the α subunits, phosphorylation of β subunits has been observed at both tyrosine and serine residues (95, 96), although the precise role of this phosphorylation is uncertain at present. The EGF receptor, a 170-kD glycoprotein (97), exhibits ligand-induced tyrosine kinase activity (98). Sequence comparison of the EGF receptor with the insulin receptor reveals significant homology among different regions of the two receptors (97) and suggests that at least portions of these molecules were derived from the same ancestral gene.

IgA and its receptor. IgA is produced by plasma cells as either a monomer (160 kD) or a polymer covalently linked by a 15-kD junctional (J) protein, polymeric IgA, is found in all external secretions, including bile, saliva, and tears (99). Polymeric IgA in exocrine fluid such as bile differs from serum polymeric IgA by the presence of an associated glycoprotein, termed secretory component, a portion of the IgA receptor.

The IgA receptor is a transmembrane glycoprotein found on the sinusoidal surface of the hepatocyte plasma membrane (100, 101). This receptor differs from most other endocytotic receptors in that it does not recycle. The mature IgA receptor has a molecular mass of about 120 kD (102). Polymeric IgA binds to its receptor via a monovalent bond (103). After initial endocytosis, polymeric IgA is transported across the hepatocyte and is secreted intact into bile along with secretory component, upon proteolysis of the receptor (99, 104).

Lysosomal enzymes and their receptors. In most cells the sorting of newly synthesized acid hydrolases to lysosomes is mediated by the phosphomannosyl recognition system (10). Newly synthesized lysosomal enzymes undergo cotranslational glycosylation which includes the addition of the phosphomannosyl recognition marker, Man-6-P. The lysosomal enzymes marked with Man-6-P bind to their receptor in the Golgi. These ligand-receptor complexes are then concentrated and bud from

the Golgi in coated vesicles with subsequent transport to lysosomes.

Two distinct Man-6-P receptors have been identified and characterized, the 215-kD cation-independent receptor and the 46-kD cation-dependent receptor (10, 105). The 215-kD receptor also binds IGF-II (10). Both receptors bind Man-6-P with similar affinity (106). Because exogenously added Man-6-P does not affect the lysosomal enzyme levels of cells, the newly synthesized enzymes must be directed to lysosomes via an intracellular route (107). However, there is considerable evidence that the 215-kD receptor functions in both intracellular sorting of lysosomal enzymes as well as uptake of extracellular lysosomal enzymes. The 46-kD receptor, on the other hand, only participates in intracellular lysosomal enzyme sorting under physiologic conditions (10).

LRP/α_2-macroglobulin receptor and its ligands. A LRP of 600 kD (composed of a 515-kD subunit and a 85-kD subunit) has recently been identified and characterized as a new member of the LDL receptor family (108, 109). This plasma membrane receptor is unique not only because of its large size but also because of its multifunctional nature in ligand recognition: LRP mediates the binding and endocytosis of several structurally and functionally distinct ligands including apolipoprotein E-enriched β-VLDL (110), activated α_2-macroglobulin (111), t-PA either in its free form (112) or complexed with PAI-1 (113), and u-PA·PAI-1 complex (114, 115). A 39-kD receptor-associated protein which copurifies with LRP has been shown to modulate ligand binding to this receptor (116–119). This receptor-mediates ligand endocytosis into the liver *in vivo* (120) and *in vitro* (116, 119), suggesting that this receptor may function as a general clearance receptor *in vivo* to clear plasma proteins and lipoproteins.

STRUCTURE OF CELL SURFACE RECEPTORS

The molecular cloning of cDNAs encoding endocytotic receptors has enhanced our understanding of receptor structure significantly including new insights into structure-function relationships. Certain structural features appear to be common among all endocytotic receptors which use the coated pit pathway (Fig. 5). For example, each receptor contains an extracellular ligand binding site(s), a hydrophobic transmembrane helical structure, and a cytoplasmic tail containing endocytosis and other functional signals (121). Depending upon their orientation within the plasma membrane, two classes of receptors are evident. Type I receptors are arranged with their amino termini on the extracellular side of the membrane (*e.g.* receptors for LDL, IgA, EGF, insulin, α_2-macroglobulin, and Man-6-P) (83, 122). Membrane insertion in this category is analogous to the transmembrane transfer of secretory protein across the endoplasmic reticulum and involves cleavage of a signal recognition sequence. Type II receptors, on the other hand, are synthesized without a cleavable amino-terminal signal sequence and inserted into the plasma membrane with their carboxyl termini in the extracellular milieu (*e.g.* receptors for transferrin and ASGP). The receptor structures and some of their modifications are illustrated in Figure 5.

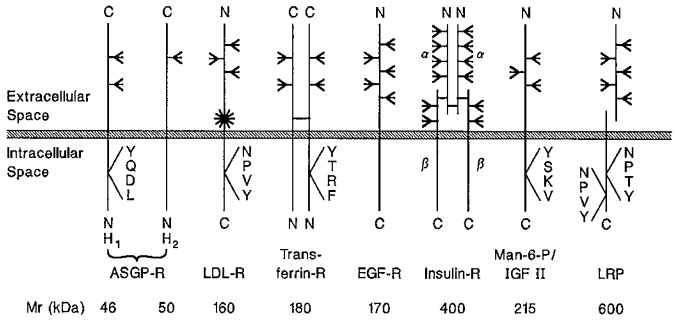

Figure 5. Comparison of representative endocytotic receptors. The orientation of each receptor is indicated (*N*, amino-terminal end; *C*, carboxyl-terminal end). The transferrin receptor is shown as a disulfide-linked homodimer; and the insulin receptor is shown as a disulfide-linked heterotetramer. The amino acid motifs responsible for endocytosis are indicated for several well characterized receptors. The sizes of the receptors are not drawn to scale; however, their molecular mass are given underneath each receptor. The representative sites of *N*-linked oligosaccharides are indicated by *fork* symbols; and the site of *O*-linked sugar on the LDL receptor is indicated by a cluster of *horizontal lines*.

Ligand binding domains. Each receptor contains at least one ligand binding domain on its extracellular region. However, the nature of ligand-receptor interactions is not necessarily the same for each receptor. Among those receptors whose ligand binding domains have been studied, the LDL receptor is one of the best characterized. The ligand binding domain of the LDL receptor contains seven cysteine-rich repeats with each repeat sharing high homology with the cysteine-rich region of complement component C9 (84, 123). A striking feature of each repeat sequence is the presence of a cluster of negatively charged amino acids. These sequences are complementary to the positively charged sequences in the well characterized ligands for LDL receptor, apolipoprotein E (124), and apolipoprotein B (125).

Similar cysteine-rich complement-type repeats are also found in LRP/α_2-macroglobulin receptor (108). The large subunit of the LRP molecule is arranged such that it resembles four LDL receptors. As mentioned above, LRP is a multifunctional receptor with several structurally and functionally distinct ligands. Because these ligands (*e.g.* β-VLDL, α_2-macroglobulin, t-PA) do not compete with one another for binding (112), they are likely to bind to different sites on the LRP molecule.

Membrane spanning domain. This domain, among different receptors, normally consists of a stretch of about 20 hydrophobic amino acid residues. The hydrophobic nature of this region determines the high affinity for the lipid bilayer of the plasma membrane. It appears that the hydrophobicity of these amino acids is the major determinant, because of the 22 amino acids of the LDL receptor transmembrane region, seven differ between human and cow, but all of the substitutions are hydrophobic (83). The function of this membrane spanning domain is to anchor the receptor through the plasma membrane.

The cytoplasmic tail. The cytoplasmic tails of endocytotic receptors generally contain crucial information essential for receptor function. Attempts to define the molecular elements responsible for receptor-mediated uptake and intracellular sort-

ing signals have been accelerated by the elucidation of receptors' primary sequences and the development of mutagenesis techniques. The lack of conserved primary sequence among these receptors suggests that they do not associate with coated pits/vesicles via a common primary recognition sequence. However, signals for receptor clustering are beginning to emerge for several individual receptors.

An endocytosis signal for the LDL receptor was first derived from patients with homozygous familial hypercholesterolemia (83, 126). Sequence analysis has shown that the tetrameric sequence, NPXY, is required for efficient coated pit-mediated internalization of the LDL receptor (127, 128). The signal for rapid internalization of the Man-6-P/IGF II receptor has been localized to the sequence YSKV in the receptor cytoplasmic tail (129, 130). Detailed analysis of this motif revealed that the essential elements are an aromatic residue, especially a tyrosine, separated from a large hydrophobic residue by two amino acids (130). On the other hand, YXRF was identified as the internalization sequence for the transferrin receptor (131). Recent studies on the three-dimensional structures and functions of several cytoplasmic domains strongly support the notion of an endocytosis motif which exists as a "tight turn" and contains an aromatic amino-terminal residue and either an aromatic or large hydrophobic carboxyl-terminal residue (131, 132).

The position of the usual 4-amino acid internalization motif does not seem to be important. For example, the YSKV motif for the Man-6-P/IGF II receptor can be moved to a more proximal region of the cytoplasmic tail with only a modest loss of activity (130, 131). Therefore, any conformational determinant must localize to the 4-amino acid motif itself. Taken together, effective internalization signals fall into a general structural recognition motif rather than a specific amino acid sequence (130–132).

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