Journey to the Center of the Cell: Role of the Receptosome

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Summary. Fibroblasts contain a specific internalization pathway that carries hormones as well as some proteins and viruses from the cell surface to the cell interior. Initially, the ligands bind to mobile receptors that are randomly distributed on the cell surface. Next the ligand-receptor complexes are trapped and concentrated in specialized regions of the membrane termed bristle-coated pits. From the pit a smooth-walled vesicle containing the ligand forms and carries the ligand to the cell interior. Because of its role in receptor-mediated endocytosis, this vesicle has been termed a "receptosome."

Almost every science student remembers the first time he looked through a microscope at a drop of pond water and saw a wide variety of organisms actively swimming about. However, few of us realize that equally frenetic activity is taking place in the cytoplasm of a cell. Mitochondria swim from place to place like tiny snakes. Lysosomes and other small vesicles dance about in purposeful motion that has been called saltatory motion, from the Latin *saltare* (to dance). The purpose of this motion is to move molecules contained within the vesicles from one place in the cell to another in order to keep the cell functioning.

All cells must bring biologically important molecules from the cell exterior to the interior. Small molecules are carried through the membrane by specific transport systems. Large molecules such as hormones and plasma proteins, as well as toxins and viruses, are ingested by another route. Phagocytosis and pinocytosis have been used to describe the process by which cells ingest solids or liquids. Although these have been useful terms, they do not distinguish between the functionally and morphologically distinct pathways that have recently been recognized and are the topic of this article.

Receptor-Mediated Internalization

On the surface of fibroblasts, embedded in the membrane, are a wide variety of specific receptors for biologically active molecules. These include receptors for insulin, epidermal growth factor (EGF), somatomedin C, platelet-derived growth factor (PDGF), low-density lipoprotein (LDL), α_2-macroglobulin (α_2M), triiodothyronine (T_3), diphtheria toxin, shigella toxin, pseudomonas toxin, lysosomal enzymes, and thrombin (*1–3*). Many viruses also bind to specific cellular receptors. The location of a few of these receptors has been studied by electron microscopy. The receptors for α_2M, EGF, pseudomonas toxin, and some viruses are diffusely distributed on the cell surface. We assume that most of the other receptors are also randomly distributed except for LDL receptors, most of which have been reported to be preclustered in coated pits (*3–8*). After ligands bind to their receptors, the ligand-receptor (L-R) complexes move to specialized regions of the membrane where the complexes form clusters. These specialized regions are bristle-coated pits; the bristle coat is made from the protein clathrin (*9*).

Coated pits were first observed by Roth and Porter in the plasma membrane of mosquito oocytes (*10*) and were later shown to occur in other cell types. They noted that the coated pits were filled with yolk protein and proposed that these pits were involved in "selective adsorption" from the extracellular space. Later, some types of viruses were observed in the coated pits of fibroblasts prior to cellular entry (*11*).

During a study of the metabolism of LDL in cultured fibroblasts, ferritin-labeled LDL was observed in coated pits prior to cellular entry, and subsequently α_2M, EGF, pseudomonas toxin, and a lysosomal enzyme (β-galactosidase) were also seen clustered in coated pits prior to entry; indirect evidence indicates that insulin and triiodothyronine also cluster in coated pits (*4, 6, 7, 12–16*). Thus, as suggested by Roth and Porter (*10*), coated pits are an entry route for many different ligands interacting with physiologically important receptors.

Fluorescence Studies

Because most ligands initially bind to receptors that are located outside of coated pits, there must be a mechanism to move L-R complexes to coated pits where they cluster. This is accomplished by random diffusion of the complexes in the plasma membrane. The movement of proteins embedded in membranes can be measured by photobleaching methods, some of which employ laser microscopy of fluorescently labeled lipids or proteins (*17*). That method has been used to measure the mobility at 23°C in the plasma membrane of the L-R complexes of α_2M, EGF, insulin, and T_3. All have mobilities from 3×10^{-10} to 9×10^{-10} square centimeters per second. This range approximates that for most membrane proteins and indicates that the L-R complexes diffuse about randomly in the lipid bilayer. If we assume that coated pits occupy 2 percent of the surface area of a cell, then each receptor will encounter a coated pit every 4 to 5 seconds (*18*).

Because of the small number of receptors on the cell surface, the binding of fluorescently labeled ligands to the cell membrane and their subsequent entry has been difficult or impossible to detect by means of standard fluorescence microscopy. The development of video intensification microscopy (VIM) in which a silicon intensifier target TV camera is substituted for the eyepiece of a fluorescence microscope has made the continuous observation and recording of various fluorescent ligands possible (*19*). This simple system greatly amplifies the fluorescent signal so that only very low levels of light are needed. As a consequence, there is a marked decrease both in the bleaching of the fluorescent probe and in the light-induced damage to intracellular structures that arrests intracellular motion and can kill cells.

If rather high concentrations of ligand

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are used to saturate cellular receptors, ligands can be seen covering the cell surface when binding is carried out at 4°C to prevent internalization (Fig. 1A). The ligand whose surface binding is most easily visualized is α_2M; this is because fibroblasts have a large number of surface receptors for α_2M (about 600,000 per cell) and 15 to 20 rhodamine molecules can be attached to each α_2M molecule without affecting its specific cellular binding (*2, 4, 20, 21*). Images of fluorescent derivatives of EGF, insulin, and triiodothyronine, which have fewer cellular receptors or to which fewer fluorescent molecules can be attached, have the same general appearance but are much weaker in intensity (*14, 15, 22*).

When cells are warmed to 37°C for a few minutes, the amount of fluorescent ligand on the cell surface falls, and small bright spots appear in the cytoplasm; these are intracellular vesicles containing ligand (Fig. 1D). These vesicles move about in the cytoplasm by saltatory motion, and gradually over a 30- to 60-minute interval, depending on the cell type, accumulate in the center of the cell next to the nucleus where the Golgi apparatus is located. Fluorescence double-labeling in which rhodamine is attached to one ligand (such as EGF) and fluorescein to another (such as α_2M) has been used to show the very same vesicles can contain all the different ligands (α_2M, insulin, EGF, triiodothyronine, and LDL). This result also shows that there is a single type of vesicle that is used by all ligands, and it excludes the possibility that each ligand enters in a different type of vesicle (*14, 15, 23*).

Receptosomes—The Invisible Vesicles

In fibroblastic cells, lysosomes, because of their high protein content, are readily detected by their dark appearance when examined by phase contrast optics. When the fluorescently labeled vesicles containing recently internalized α_2M, EGF, insulin, or T_3 were examined by phase-contrast microscopy, the vesicles were not "phase-dense" and, therefore, probably not lysosomes. In fact, the fluorescent vesicles could not be seen at all when viewed by phase contrast microscopy, an indication that the vesicles and the surrounding cytoplasm have about the same concentration of protein. It required electron microscopy to reveal the characteristic structure of these vesicles, which we had termed "receptosomes," to indicate their role in receptor-mediated endocytosis (*14, 15, 19, 24, 25*).

Electron Microscopy

α_2-Macroglobulin is an ideal molecule to use for electron microscopic studies because, in addition to the large number of α_2M molecules taken up by fibroblasts, its large size allows it to be attached to electron opaque markers without affecting its specific cellular binding. Thus, a large number of clear images of α_2M entering cells can be readily obtained. Initially, α_2M is seen bound to receptors that are diffusely distributed on the cell membrane. However, after α_2M binding, the α_2M-receptor complexes begin to cluster in coated pits, even at 4°C (Fig. 2A). Clustering in coated pits at 4°C is not observed with all L-R complexes; for example, EGF and some viruses bound to their receptors do not

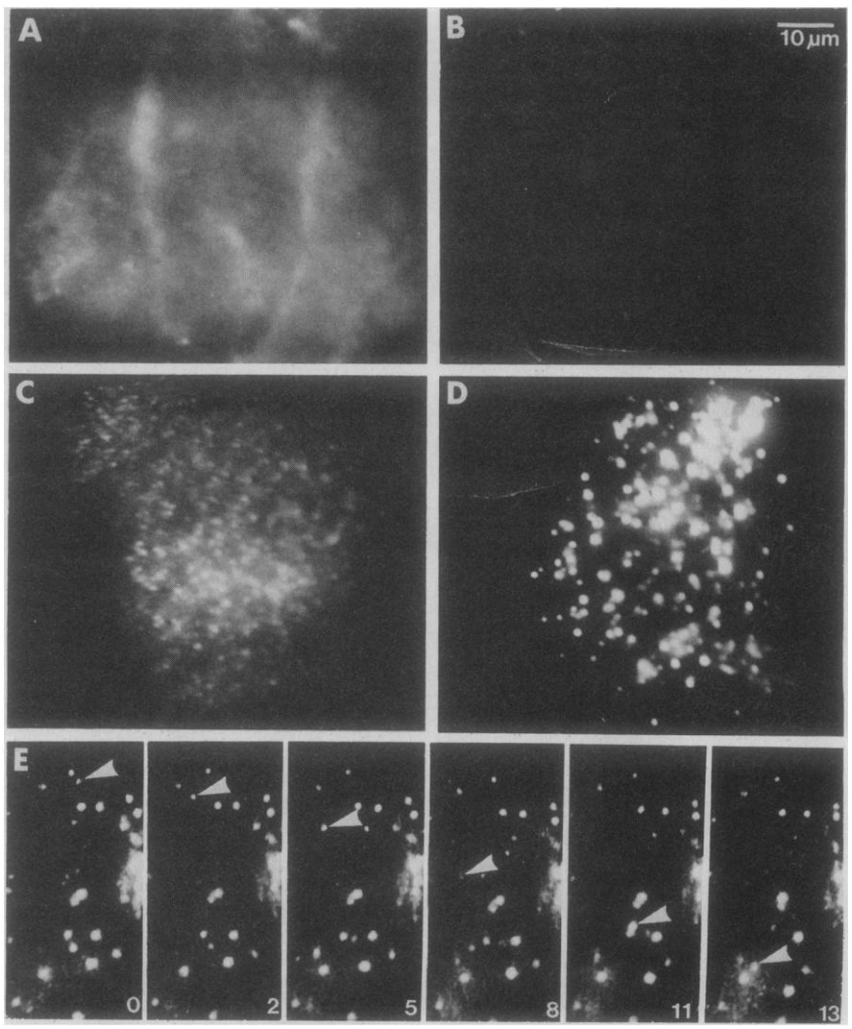


Fig. 1. Fluorescence images of Swiss 3T3 cells incubated with rhodamine-labeled α_2-macroglobulin (Rα_2M). Swiss 3T3 cells were incubated at 4°C in serum-free medium for 60 minutes with (A) Rα_2M (1 mg/ml), (B) Rα_2M (1 mg/ml) with unlabeled α_2M (5 mg/ml) (B), or (C, D, and E) Rα_2M (100 μg/ml). Cells were either fixed at 4°C with 3.7 percent formaldehyde in phosphate-buffered saline (PBS) (with Ca^{2+}, Mg^{2+}) (A, B, and C) or warmed to 37°C for 5 minutes (D and E) and then either viewed directly at 37°C (E) or fixed in 3.7 percent formaldehyde (D). (A) demonstrates the diffuse appearance of Rα_2M binding at 4°C in saturating amounts of Rα_2M, and the competition of this fluorescence pattern by unlabeled α_2M (B). The bright linear pattern represents the edges of a single cell, three of which are seen adjacent to each other in (A). At lower concentrations of Rα_2M, weak small spots corresponding to clustered Rα_2M in coated pits on the cell surface can be seen (C). However, on warming to 37°C, the endocytosis of Rα_2M into receptosomes produces a much brighter punctate pattern in the cytoplasm (D). The pattern in (C) is known to be clustered ligand in surface-coated pits by the ability to remove this pattern by external agents such as pepsin, the colabeling of these structures in double-label experiments with antibody to clathrin, and the visualization of these as surface-coated pits in parallel electron microscopic experiments. The pattern in (D) represents intracellular receptosomes through the use of similar experimental methods (*2, 4, 14, 15, 19–21, 23–25, 33*). In living cells the saltatory motion of these receptosomes can be seen in (E) which shows single-frame images from a video recording. The numbers in the lower right corner indicate the number of seconds at the beginning of the sequence. The white arrows point out the position of a single rapidly saltating receptosome (video intensification microscopy, ×760).

cluster readily at 4°C. Within 2 minutes after cells kept at 4°C are raised to 37°C, α_2M is found inside the cell in an uncoated vesicle, the receptosome. A newly formed receptosome is about 2500 angstroms in diameter and is often seen near a coated pit (1500 Å in diameter), suggesting that it has formed from the coated pit (see below). Receptosomes have a characteristic appearance that allows them to be recognized even when they do not contain a ligand (see Fig. 2B). Receptosomes range in size from 2500 to 4000 Å in diameter. They have a smooth continuous membrane. Attached to their inner surface is a proteinaceous layer probably representing L-R complexes. The centers of receptosomes appear empty. They also have two other characteristic features. In appropriate preparations, a single small intraluminal vesicle about 600 Å in diameter is observed, and also one edge of the receptosome is often thickened and has a frill-like appearance. Receptosomes are often surrounded by small tubular or round vesicular profiles, and some images suggest receptosomes may be in continuity with these vesicular structures.

Receptosomes have been shown by various electron microscopic studies to contain α_2M, EGF, LDL, β-galactosidase, vesicular stomatitis virus (VSV), and adenovirus (*6, 16, 23, 25*). An example of a receptosome containing both VSV and α_2M is shown in Fig. 2C. The name receptosome (receptor-body) was chosen to indicate that ligands entering the cell via specific receptors appear in this organelle. The presence of viruses and other ligands in intracellular vesicles has been observed by various workers (*6, 8, 12, 25–28*). Only recently has it been appreciated that the first vesicle in which the ligand appears after cellular entry is a special organelle and not some type of lysosome (*25, 27*). Materials that do not bind to specific receptors mainly enter cells by nonspecific endocytosis in another type of organelle termed a pinosome. Within a few minutes of their formation pinosomes fuse with lysosomes and their contents begin to be degraded (*4, 29*). There are two types of pinosomes: small micropinosomes (~0.08 micrometers in diameter) that are derived from surface caveolae, and large macropinosomes (~2 μm in diameter) formed when surface ruffles fall back and fuse with adjacent areas of the cell surface. Both of these organelles have a clear lumen and are commonly surrounded by actin microfilaments. They have a regular, smooth, uninterrupted membrane and lack the fuzzy peripheral lamellar structure or intraluminal vesicles characteristic of receptosomes.

Receptosomes containing α_2M arrive in the Golgi region 10 to 30 minutes after entry, depending on the cell type. Between 15 to 60 minutes the ligand leaves the receptosome and begins to accumulate in lysosomes. About 15 to 30 minutes after cellular entry α_2M, EGF, and LDL begin to degrade and this roughly corresponds to the time at which they appear in lysosomes. Unlike some other ligands, α_2M is not totally degraded soon after its entry into lysosomes. How receptosomes transfer their contents to the Golgi–Golgi endoplasmic reticulum lysosome complex (GERL) system and then on to lysosomes is unknown. Receptosomes probably fuse selectively with certain regions of the Golgi-GERL system. They can also fuse with each other but never fuse with other intracellular organelles such as mature lysosomes, macropinosomes, or elements of the endoplasmic reticulum. Fusion of receptosomes with the Golgi-GERL system must require the presence of a specific recognition system between receptosomes and the Golgi.

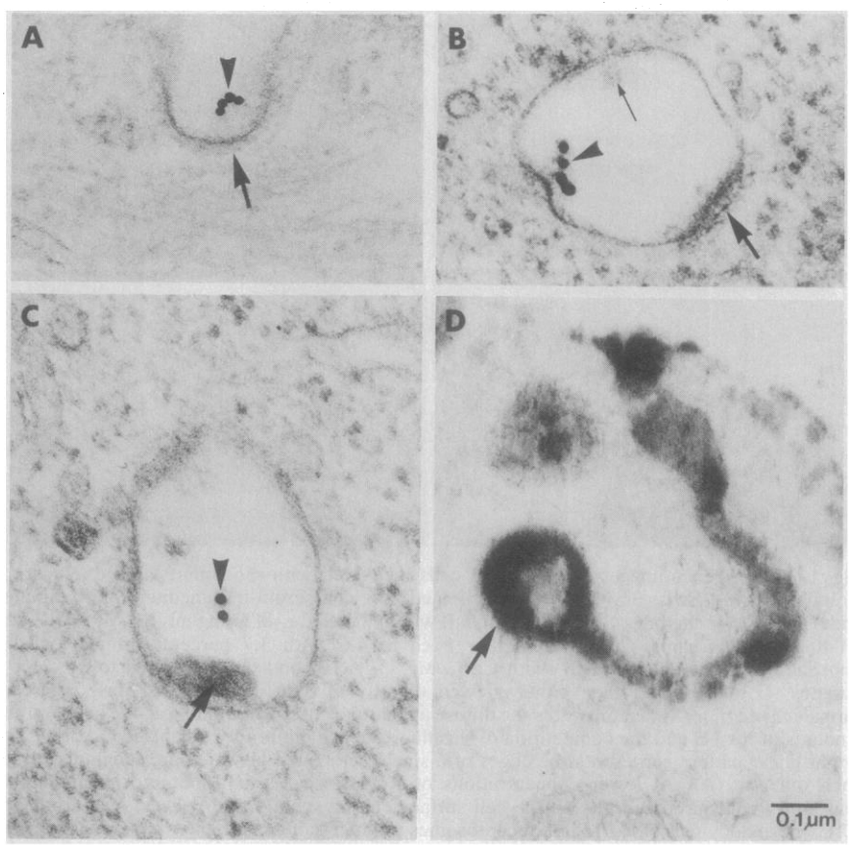


Fig. 2. Electron microscopic appearance of α_2-macroglobulin (α_2M) internalization. Swiss 3T3 cells were incubated at 4°C in SFM with α_2M labeled with colloidal gold (Auα_2M). When fixed in glutaraldehyde at 4°C (A), the Auα_2M (arrowhead) is clustered in coated pits (arrow). After being warmed to 37°C for 5 minutes prior to fixation (B), Auα_2M (arrowhead) is seen in a receptosome, which also shows a thickened edge (arrow) and a single small intraluminal vesicle, shown here in a glancing section (small arrow). Cells that had been incubated in Auα_2M, as well as vesicular stomatitis virus (VSV), show co-internalization of Auα_2M (arrowhead) and VSV (arrow) into the same receptosome (C). Cells that had been labeled at 4°C with concanavalin A and peroxidase and then warmed to 37°C for only 30 seconds show convoluted extensions of coated pits (arrow) connected to the cell surface, here labeled with the peroxidase reaction product (D) (×80,000).

Formation of Receptosomes

Two models have been proposed to account for how an uncoated vesicle (the receptosome) forms from the coated pit. Because in the original study of Roth and Porter (*10*), and in later studies on fibroblasts, images of what appeared to be coated vesicles containing ligand lying close to the plasma membrane were observed, the conclusion was drawn that coated pits pinched off to form coated vesicles (*4, 8, 10, 25, 30*). It was postulated that at some later stage coated vesicles lost their coats and the coat protein, clathrin, recycled to the cell surface. This idea was supported by the ease with which clathrin baskets could be assembled and disassembled in cell-free extracts (*31*). Another possible interpretation of these images of coated vesicles lying close to the plasma membrane is that they represent tangential sections

through deep coated pits. Because uncoated vesicles can be seen forming from the necks of coated pits, we suggested that coated pits are stable structures in the plasma membrane of fibroblasts and that one role of the coat protein clathrin is to serve as a skeleton to maintain the coated pit in communication with the cell exterior (*25, 32*). To distinguish between a pinching-off recycling model and one in which uncoated vesicles form directly from the coated pit, two types of experiments have been performed. In one set of experiments, it has been directly demonstrated with the use of impermeant electron opaque labels that what appear to be coated vesicles are really tangential sections of coated pits connected to the cell surface by long, often tortuous, necks (Fig. 2D). In other experiments, we have used another new technique: microinjection of proteins into single cells. Antibody to clathrin has been injected into fibroblasts and then the ability of these cells to carry out endocytosis of α_2M was studied (*33*). When excess antibody to clathrin is injected into the cells, we observe that all the coated pits bind antibody and that the excess antibody remains free in the cytoplasm. This free antibody is still active since microinjection of clathrin into a cell previously injected with excess antibody to clathrin resulted in large precipitates. Even in the presence of excess antibody to clathrin, endocytosis of α_2M is entirely normal. Because the injected antibody should aggregate free clathrin or clathrin-coated vesicles, we conclude that free clathrin or clathrin-coated vesicles are not formed during the endocytosis process and that coated pits are stable elements attached to the plasma membrane. These experiments also show that antibody to clathrin can bind to those regions of the clathrin molecules exposed to the cytoplasm without dissociating clathrin from the coated pit or interfering with the biochemical function of clathrin.

Inhibitors of Internalization and the Transglutaminase Hypothesis

All forms of endocytosis are energy-requiring (*34*). In addition, some compounds selectively inhibit the receptor-mediated pathway. Among these are primary amines. The most effective of these is dansylcadaverine, which inhibits the internalization of α_2M, insulin, T_3, EGF, LDL, and pseudomonas toxin (*2, 23, 35*). Analysis by electron microscopy of the site at which dansylcadaverine and related inhibitors block internalization showed that α_2M, pseudomonas toxin, and LDL were diffusely bound to the cell membrane and unable to form clusters in coated pits. This inhibition of clustering results in a decreased rate of internalization (*2, 23, 35*).

Table 1. Effect of various inhibitors on ligand internalization. (+) Inhibits; (−) does not inhibit.

Ligand	Dansylcadaverine	BDONV	Bacitracin
α_2M	+	+	+
T_3	+	+	+
Insulin	+	+	+
EGF	+*	−	−
LDL	+	NT	−
Pseudomonas toxin	NT†	NT	NT
VSV	+	NT	−

*Active at high concentrations. †Methylamine is active.

A possible biochemical explanation for the mechanism of amine inhibition came from the realization that amines were potent inhibitors of transglutaminase, a widely distributed type of enzyme (*36*). This enzyme is capable of cross-linking proteins together; for example, a lysine of one protein and a glutamine of another can form an isopeptide bond (R·NH·CO·R). Primary amines are analogs of lysine and act as competitive inhibitors of the enzyme. Conceivably, receptor-ligand complexes might be held in place in coated pits by the actions of such an enzyme, which induces the cross-linking of the receptor to some other protein component of the coated pit. To examine one part of this hypothesis we looked at other transglutaminase inhibitors to see whether they blocked α_2M entry. Using analogs of lysine and glutamine as well as *N*-benzyloxycarbonyl-5-diazo-4-oxonorvaline *p*-nitrophenyl ester (BDONV) (an affinity label that irreversibly inactivates transglutaminases), we have found a good correspondence between the concentration of those compounds required to inhibit the internalization of α_2M, and their ability to inhibit transglutaminase. However, when we extended this type of analysis to different ligands, a subgrouping in specificity was revealed (Table 1). The primary amine, dansylcadaverine, inhibited the uptake of all ligands studied although EGF was relatively insensitive, requiring rather high concentrations of dansylcadaverine to block entry. Bacitracin affected some of the ligands but not others (*37*). There has not yet been extensive testing of BDONV. The inhibition studies show variation in the biochemical mechanism that induces clustering of different ligands. This variation could allow the cell to independently regulate the rate of clustering and internalization of different classes of ligand.

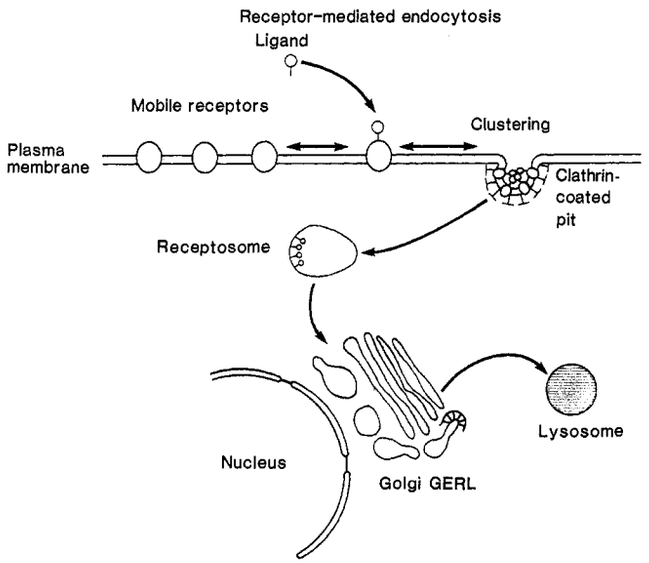
Viral Entry

In addition to soluble ligands, many viruses enter cells by coated pits and are found in uncoated vesicles. These include adenovirus, vesicular stomatitis virus (VSV), Rous sarcoma virus, and Semliki forest virus (*8, 26, 38, 39*). These viruses are so large that one virus can fill a pit. To be certain that viruses and soluble ligands enter cells by the same route, we have done experiments using electron microscopy and showed VSV or adenovirus in the same pit or receptosome as α_2M or EGF. An example of a receptosome containing α_2M molecules and a VSV particle is shown in Fig. 2C. Amantadine and rimantadine are two antiviral compounds that act by inhibiting viral penetration. These are tricyclic compounds with a primary amine moiety that is important for antiviral activity. Rimantadine and amantadine are also inhibitors of fibroblast transglutaminase and inhibit the uptake of ligands such as α_2M. Conversely, dansylcadaverine is a very potent inhibitor of VSV internalization, about 20-fold more potent than rimantadine (*40*). Because dansylcadaverine is a very potent inhibitor of virus internalization, it and related compounds are being evaluated for their usefulness as antiviral agents.

Uptake and Binding Studies

Fluorescence and electron microscopy are useful in that they show where ligands are in a cell, but these techniques are not suitable for quantifying such interactions. ^{125}I-Labeled α_2M and other ligands have been used to quantify the interactions of these ligands with cells or cell membranes (*20, 21, 38, 41*). Measurements of α_2M uptake indicate that each cell can internalize up to 1.5×10^6 α_2M molecules per minute. Electron microscopy indicates that all uptake occurs via coated pits. Therefore, by knowing the number of coated pits per cell (~1000) and the number of α_2M molecules detected in a coated pit (~20), we can determine the number of times a coated pit fills and empties in a minute. We calculate that there are up to three cycles of internalization each minute (*20*).

Using ^{125}I-α_2M, we have detected two classes of binding sites: a very high affinity class (K_d 0.1 n*M*) and a lower affinity class (K_d 100 n*M*). There are about 10,000 high affinity and 600,000 low affinity sites per cell. One conspicuous finding has been that the compounds that inhibit clustering in coated pits (dansyl-



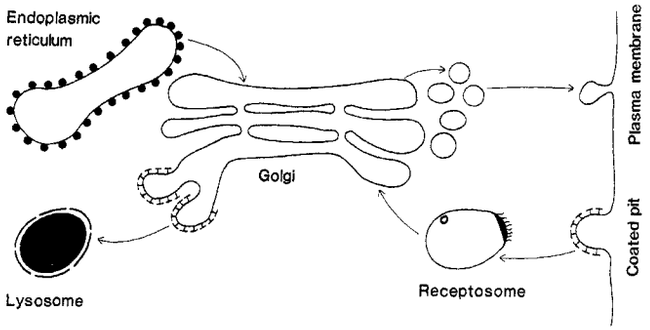


Fig. 3 (left). Summary of steps involved in the binding, clustering, and entry of a typical ligand into a fibroblast. Fig. 4 (right). Summary of some possible pathways of the traffic of macromolecules.

cadaverine, bacitracin, and BDONV) all inhibit the high affinity binding component but not the lower affinity one. It seems possible that the low affinity binding represents α_2M bound to diffusely distributed receptors ($\alpha_2M–R_L$), and the high affinity binding represents clustered α_2M-receptor complexes ($\alpha_2M–R_H$) in coated pits:

$$\alpha_2M + R \leftrightharpoons \underset{\text{(diffuse)}}{\alpha_2M–R_L} \leftrightharpoons \underset{\text{(clustered)}}{\alpha_2M–R_H}$$

This clustered high affinity state of α_2M-receptor complexes would require the active form of the transglutaminase-like enzyme. More than one class of binding sites has been found for many hormone receptor complexes (*1–3*). Whether these interactions are affected by dansylcadaverine, BDONV, or bacitracin has not been examined.

The Directionality Problem and Saltatory Motion

In their journey from the cell surface to the interior, receptosomes can move halfway across a cell in a few minutes (Fig. 1E). Microtubules radiate out from the perinuclear region toward the cell surface where receptosomes are formed. The motion of receptosomes appears to be guided in short straight segments toward the cell center. This is probably due to their association with microtubules, because the microtubule poison colchicine specifically arrests their motion. This combination of saltatory motion and microtubular guidance appears to bring receptosomes into the perinuclear region. In fibroblasts, the Golgi apparatus lies near the center of the cell adjacent to the nucleus. The Golgi contains a system of functionally and probably anatomically interconnecting stacks and cisternae where proteins are processed, addressed, and sent to their various destinations.

Special Features of the Coated Pit–Receptosome Pathway

The list of substances that enter the cell by coated pits and receptosomes is growing and will probably include other hormones, growth factors, metal-binding proteins, and viruses. Because this system rapidly and selectively concentrates L-R complexes in the coated pits prior to entry, the cell can internalize all of an L-R complex within a few minutes without internalizing significant amounts of other surface proteins.

The ligand recognition portion of each receptor is obviously different, and this is the basis of its selectivity. The fact that a wide variety of different receptors cluster in the same coated pit indicates that the receptors must have some structural feature in common. This structural feature is recognized by a component of the coated pit. This component would be an acceptor for occupied receptors. Whether this structural similarity is also that recognized by the transglutaminase-like enzyme postulated to catalyze the clustering process, or whether this activity influences another step in the internalization pathway, is not known. It is also not known whether the transglutaminase-like enzyme actually catalyzes cross-linking of two proteins, such as the receptor and a special protein of the pit, or simply holds the components together by a noncovalent interaction.

The unique feature of coated pits is the protein clathrin, which has subunits (M_r185,000) that are polymerized into a basket surrounding the pit (*9*). Clathrin has the properties of a peripheral membrane protein and may interact with one or more integral membrane proteins to form the coated pit; transglutaminase could be one of these proteins.

After entry into the receptosome, the ligand is transported to the Golgi region, but whether a receptor accompanies the ligand on this entire journey or rapidly returns to the plasma membrane is unknown. EGF receptors are strongly down-regulated and many may shuttle to the lysosome to be destroyed. Receptors for α_2M show no down regulation and somehow return to the surface intact. Receptors for LDL and insulin are intermediate in their behavior (*20, 42*). Presumably this pathway has the flexibility needed for such diverse regulation. Recently we have detected the phosophomannosyl receptor in receptosomes using antibodies to the receptor (*43*).

The GERL region is believed to be the site where new lysosomes are formed and lysosomal enzymes are transferred to lysosomes (*44*). We suggest that α_2M and other ligands are transferred from receptosomes to the Golgi and traverse the GERL on the way to lysosomes (Fig. 3). Using perioxidase conjugates we have recently detected EGF and β-galactosidase clustered in coated pits of the GERL, probably on their way to lysosomes (*6, 16*).

The action of many peptide hormones occurs on the cell surface. Internalization of L-R complexes can regulate the number of receptors on the surface and, thereby, the response to growth factors and hormones. Hormones such as triiodothyronine have a nuclear action and

must somehow be transferred from receptosomes to the nucleus. DNA viruses must also get to the nucleus and RNA viruses into the cytoplasm to complete their life cycle. One or more of their components probably enable virions to penetrate intracellullar membranes and to reach the cytosol and the nucleus. Toxins must also have a mechanism to reach the cytosol. It is likely that some toxins use the receptosomal pathway in this process.

Similarities Between Exocytosis and Endocytosis

In addition to internalizing molecules, cells are continuously synthesizing proteins, sending some of these to the cell surface and others to lysosomes. Because of their inaccessibility to electron microscopic and fluorescent probes, it has not been easy to trace the progress of newly formed proteins from the endoplasmic reticulum to the Golgi and from there to either lysosomes or to the cell surface. The striking morphologic similarity of coated pits in the GERL and those on the cell surface, although they differ in size, suggests they may serve similar functions. It is likely that lysosomal enzymes are concentrated in the coated pits of the GERL prior to their transfer to lysosomes and that transport of lysosomal enzymes to lysosomes requires the presence of a specific recognition marker, mannose phosphate, on the enzyme that enables it to bind to an intracellular membrane receptor (*45*). One way that lysosomal enzymes could become concentrated in the coated pits of the GERL is to use the same principles established for the internalization of L-R complexes at the cell surface.

It is possible that receptors for lysosomal enzymes are initially randomly distributed in the membranes of the Golgi. After the binding of lysosomal enzymes to these receptors, the lysosomal enzyme receptor complexes diffuse about in the Golgi membrane until they encounter and become trapped and concentrated in the coated pits of the GERL. In contrast, newly formed proteins not bound to membrane receptors of the Golgi would be excluded from this route and enter another pathway. Proteins bound for exocytosis such as the protein fibronectin are found in the lumen of the Golgi or in small uncoated secretion vesicles, but not in coated structures. Further, although it has been suggested that the virally coded G protein of VSV is secreted in coated vesicles, direct localization of G protein by electron microscopy shows that it is only present in smooth uncoated structures (*46*). A simple diagrammatic summary of these different pathways is shown in Fig. 4, which emphasizes the role of coated pits in the cell membrane and Golgi in directing proteins to their proper destination.

Conclusion

In this article, we have described some dynamic aspects of the entry of macromolecules into cells. New approaches that combine microscopy and biochemistry have begun to elucidate the nature of these processes. The results show that most unoccupied receptors are diffusely distributed on the cell surface. After ligand binding, the L-R complexes become concentrated in coated pits, and then enter the cell in a vesicle termed a receptosome. Receptosomes shuttle the ligand to the cell interior (Fig. 3). Although we have focused on the mechanism of receptor-mediated endocytosis in a single kind of cultured cell, a fibroblast, we believe these same processes occur in other cell types.

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