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Internalization of polypeptide hormones and receptor recycling

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Introduction

The insulin receptor is an integral plasma membrane glycoprotein of most cells. It consists of 2 subunits linked by disulfide bonds; the alpha and beta subunits of the receptor are synthesized by way of a single chain proreceptor which is cleaved and further processed, by the addition of complex carbohydrates, prior to insertion into the plasma membrane[49]. Recently a cDNA encoding the proreceptor has been cloned and the protein sequence

of the receptor elucidated[29, 85]. The alpha subunit of the receptor is the major binding component and the beta subunit is a tyrosine specific protein kinase which itself can be autophosphorylated.

In a separate series of events the insulin receptor complex can be removed from the cell surface by receptor mediated endocytosis. In the present review we will focus on receptor mediated endocytosis of insulin, its receptor, and the functional consequence of these events.

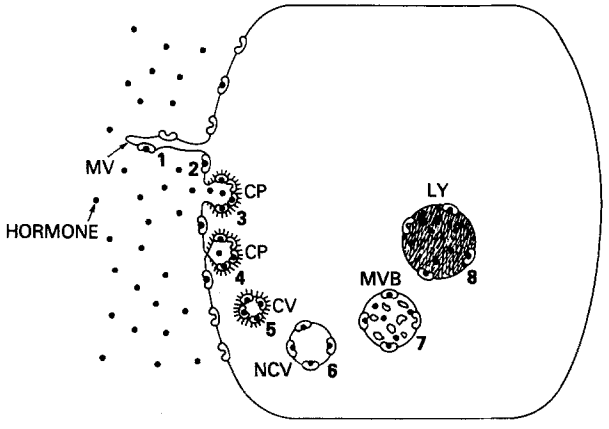

Figure 1. Schematic drawing of receptor mediated endocytosis of polypeptide hormones. Step 1 indicates initial preferential association to microvilli (MV); steps 2 and 3 represent redistribution of the hormone-receptor complex to undifferentiated plasma membrane and coated pits (CP); steps 4 and 5 represent the formation of the earliest endocytotic vesicle which is coated (CV); step 6 represents the presumed next step which involves a non-coated endocytotic vesicle (NCV); and steps 7 and 8 represent the evolution of the various lysosomal forms, i.e., multivesicular body (MCV) and various types of more dense lysosomes (LY). Reproduced from ref. 43 with permission.

Historical perspective

We now know that when ^{125}I-insulin, under physiological conditions, binds to cell surface receptors on a variety of cell types that the hormone-receptor complex is internalized by cells. Subsequently, a series of intracellular events can then separate the hormone from the receptor allowing the hormone and receptor to be processed independently. It is instructive, therefore, to remember how we arrived at this point.

Studies carried out in the late 1960s and early 1970s demonstrated that polypeptide hormones such as ACTH[63] and insulin[27, 37] could be labeled with ^{125}I in a biologically active form that would bind in a specific fashion to cells or subcellular constituents. Since these studies were carried out at low temperature to minimize hormone degradation, attention was focused on the cell surface and a fully reversible system.

Thus, in 1976 we started with the assumption that insulin bound to cell surface receptors in a fully reversible manner and that if we could develop an appropriate probe we could visualize this interaction directly at the electron microscopic level. Several electron dense probes were available, but because of problems of specificity, we settled on EM autoradiography. Though it was necessary to develop quantitative means of analysis, this methodology could be used analogously with biochemical studies. Our initial results, incubating ^{125}I-insulin with IM-9 lymphocytes, confirmed what we assumed to be true, i.e., that the hormone bound to the cell surface. Shortly afterwards, however, in collaboration with Pierre Freychet and Alphonse LeCam, we found that when ^{125}I-insulin was incubated with rat hepatocytes at 37 °C that the labeled material not only binds to the cell surface, but is extensively internalized by the cell[20]. The apparent discrepancy with the IM-9 studies was only a matter of degree since IM-9 cells do internalize insulin[13, 46], but at a slow rate and to a very small extent compared to hepatocytes[15, 17, 18].

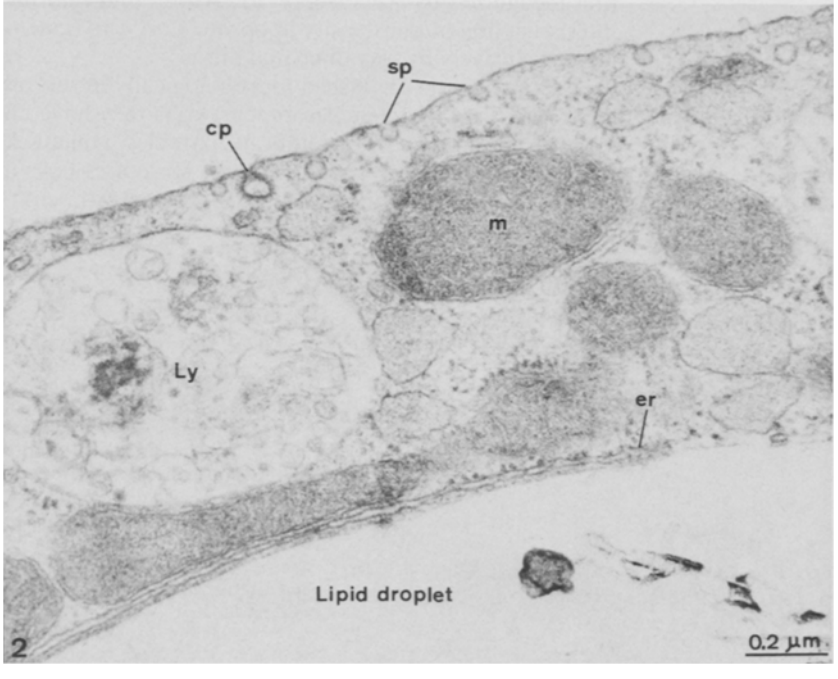

Figure 2. Low power view of the periphery of a 3T3-L1 adipocyte in culture showing a small portion of a lipid droplet, and various organelles, i.e., er, endoplasmic reticulum; m, mitochondria; ly, lysosome. The surface is relatively smooth (compare with fig. 5) but invaginates to form coated pits (cp) and small apparently uncoated invaginations (sp), × 55,000.

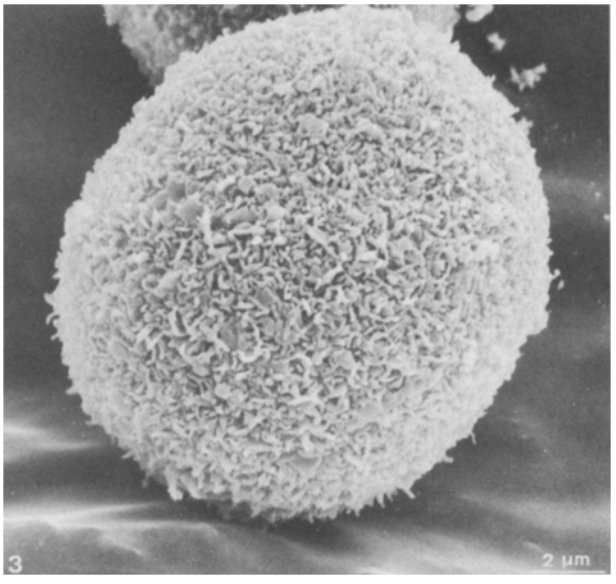

Figure 3. General view of a freshly isolated rat hepatocyte seen at the scanning microscope. The surface of the cell is filled with microvillosities and cytoplasmic projections, × 5,000.

Thus, by early 1977 it was clear that polypeptide hormones not only bound to cell surface receptors[20], but also shared post binding events with ligands as diverse as low density lipoproteins[1]. It soon became clear that many other polypeptide hormones and growth factors as well as other unrelated ligands such as α2-macroglobulin[87], asialoglycoprotein[55], transferrin[56] and others shared many common features upon interacting with cells.

Because the events related to insulin appear to be common for other polypeptide hormones, growth factors and other transport receptor proteins, we will use these examples for a broader perspective[43,44]. Finally, we will attempt to clarify certain areas of apparent controversy. The morphologic events that occur following hormone binding to receptor have been previously discussed[43,44] and are schematized in figure 1.

Events at the cell surface (steps 1, 2 and 3)

There are 3 features of the cell surface, discernable morphologically, that have been studied in detail. For some cells that bind insulin, the surface is relatively smooth (fig. 2). For many cells, however, the surface is covered with numerous microvilli; this is true for cultured human lymphocytes and freshly isolated rat hepatocytes (fig. 3). In the cultured human lymphocyte, microvilli constitute ~ 55% of the cell surface. These structures provide a large surface for initial contact with the ligand. The villi are variable in length, contain abundant cytoskeletal structures, increased density of intramembrane particles, and are largely devoid of cytoplasmic organelles[23].

Small segments of the plasma membrane are decorated with a cytoplasmic bristle coat made up predominantly of the protein clathrin[73] and at least two other minor proteins[89]. These coated segments predominate in invaginated portions of the membrane and are referred to as coated pits[1]. These pits range in size from ~ 60 nm in lymphocytes[23] and hepatocytes[17] to ~ 200 nm in human fibroblasts[1,70] and 3T3-L1 adipocytes[31,33] (figs 2 and 4). Though they occur in essentially all cells, their importance with respect to receptor mediated endocytosis was first recognized by Anderson et al., who found that the internalization of low density lipoprotein occurred essentially exclusively by way of coated pits[1].

Coated pits are distinguished morphologically in several other ways: on freeze-etched preparations they have an increased number of intramembrane particles[70]; on quick frozen deep etched preparations these structures have a basket shaped appearance comprising numerous penta-

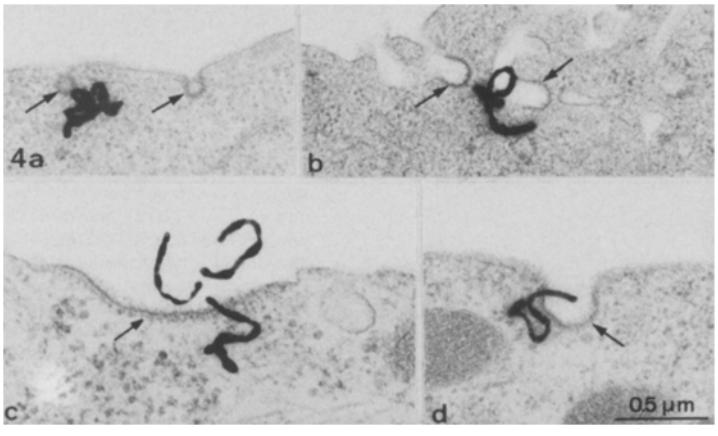

Figure 4. Coated pits (arrows) in various cell types. *a* IM-9 cultured human lymphocyte incubated with ^{125}I-insulin; *b* freshly isolated rat hepatocyte incubated with ^{125}I-monoiodo biosynthetic human insulin; *c* cultured human fibroblast incubated in the presence of ^{125}I-EGF and LDL-ferritin; *d* 3T3-L1 adipocyte incubated with ^{125}I-insulin. *a* × 23,0000; *b* × 23,000; *c* × 35,000; *d* × 25,000.

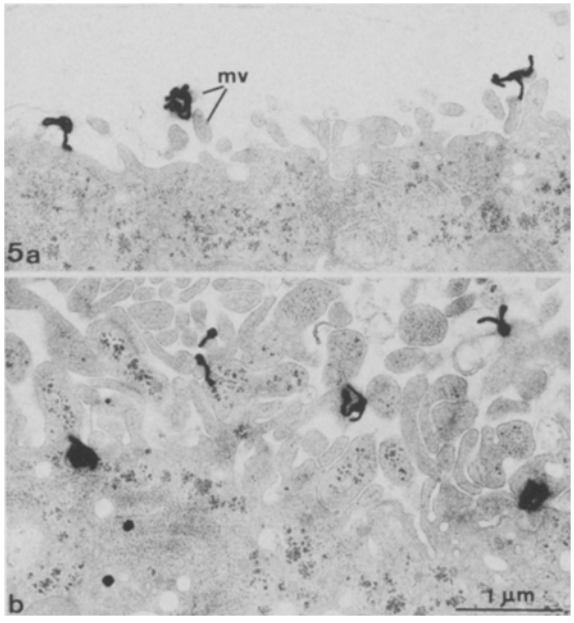

Figure 5. Periphery of freshly isolated rat hepatocytes incubated for short periods of time (2 min) at 20 °C in the presence of A-14 monoiodo biosynthetic human insulin. As seen both in section perpendicular to the plane of the plasma membrane (a) and in section tangential to this plane, autoradiographic grains are mostly associated with microvilli (mv) present on the cell surface. × 14,000.

gons and hexagons[53]. The cholesterol affinity probe, filipin, disrupts the undifferentiated portion of the plasma membrane while having little effect on coated segments[68]. The explanation for this is uncertain since filipin should have access to cholesterol on both the coated and uncoated segment and the cholesterol content is believed to be similar in both segments[81]. Regardless of the explanation, however, filipin provides a useful morphologic marker.

The remainder of the surface of most cells is comprised of a smooth trilaminar surface and apparently uncoated small invaginations. The role of the small invagination, which may be abundant in 3T3-L1 adipocytes or isolated rat adipocytes, is unclear[33].

When ^{125}I-insulin is incubated with either 3T3-L1 adipocytes, cultured human lymphocytes, or freshly isolated rat hepatocytes, autoradiographic grains localize initially and preferentially to the microvillous surface of the cell (fig. 5)[23, 31]. Preferential localization also occurs to coated pits[31] (fig. 4). With time there is a redistribution of the labeled material to the non-villous and coated portion of the membrane. This occurs under circumstances where the ligand continuously binds to receptor. Studies using both radioactively labeled[3] and fluorescently labeled ligands have shown that the ligand-receptor complex is mobile in the plane of the plasma membrane[76, 77]. These observations suggest that the hormone-receptor complex moves in the plane of the membrane from the villous

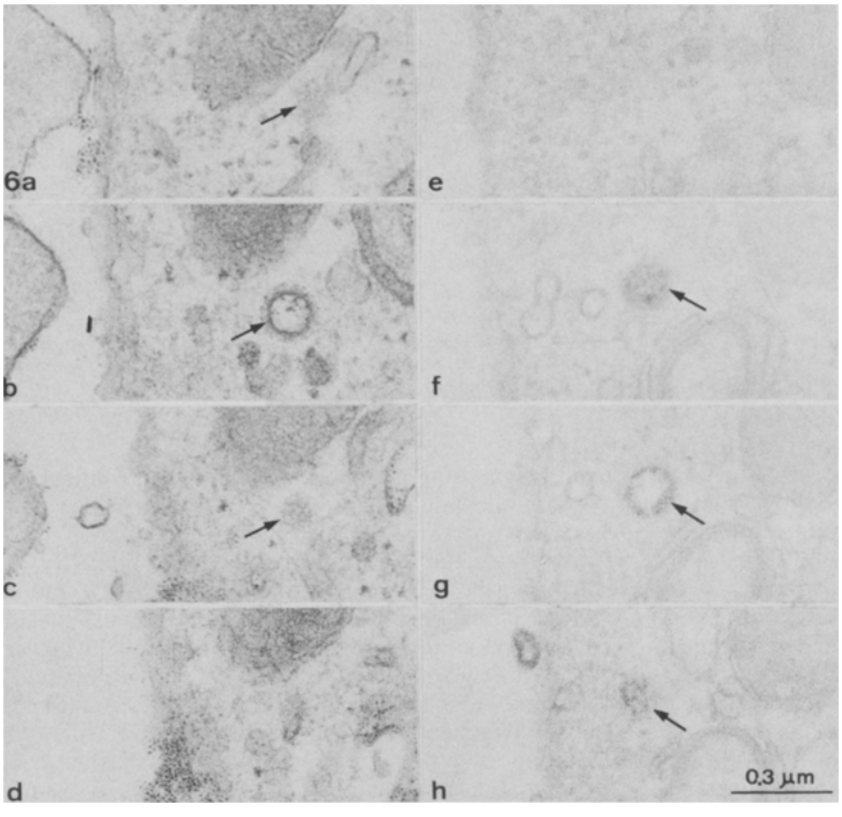

Figure 6. Serial sections through coated vesicles that have internalized cationic ferritin (a–d) or horseradish peroxidase (HRP) (e–f). As the sections progress from top to bottom, the vesicles (arrows) are identified in various planes. The examples shown here have no surface connection and are true vesicles. In the case of HRP incubation the fixed cells were stained en bloc and the sections not counterstained. × 45,000.

surface to the non-villous and coated surface; under these circumstances receptors presumably are not repleted in the villous membrane at the same rate as in other parts of the plasma membrane. These data are also consistent with the idea that the affinity for insulin may be higher in receptors in the non-villous portion of the membrane. This point is emphasized by the finding of a correlation between slowed dissociation of ^{125}I-insulin from the plasma membrane of cells and the redistribution of the ligand on the surface of the cell. Thus, when ^{125}I-insulin binds to the cell surface at low temperature and is allowed to dissociate, an increasing proportion of the ligand is redistributed to the non-villous and coated surface of the cell[11].

Initial steps of endocytosis (steps 4 and 5)

While we have indicated that internalization may occur in non-coated areas of the membrane, the process is best understood by studies of the coated pit mechanism.
Coated pits are exposed to the external environment of the cell; thus the ligand either binds initially to receptors in these structures or the complex moves into coated pits by lateral mobility. The neck of the coated pit then fuses and subsequently fissions from the membrane surface to form a coated vesicle (fig. 6). These vesicles have been demonstrated directly morphologically by serial sections which have verified that these are closed vesicular structures[31, 51, 74, 86]. The lifetime of the coated vesicle is short, i.e., 1–2 min or less and by some unknown mechanism, the coat is shed from the membrane-limited vesicle. Recent studies suggest that clathrin in coated vesicles is initially acted upon by a 70,000 mol.wt polypeptide containing ATPase activity. This uncoating ATPase presumably acts prior to clathrin release. It is of special note that this uncoating ATPase greatly prefers closed cages to free clathrin triskelions as substrates. Thus, coated vesicles are preferred to coated pits[8, 78, 79]. An alternative thesis has been proposed by Willingham and Pastan, who suggest that the coated membrane never separates from the cell surface, but instead the initial vesicle formed is uncoated[72, 88]. This controversy awaits further study for clarification.

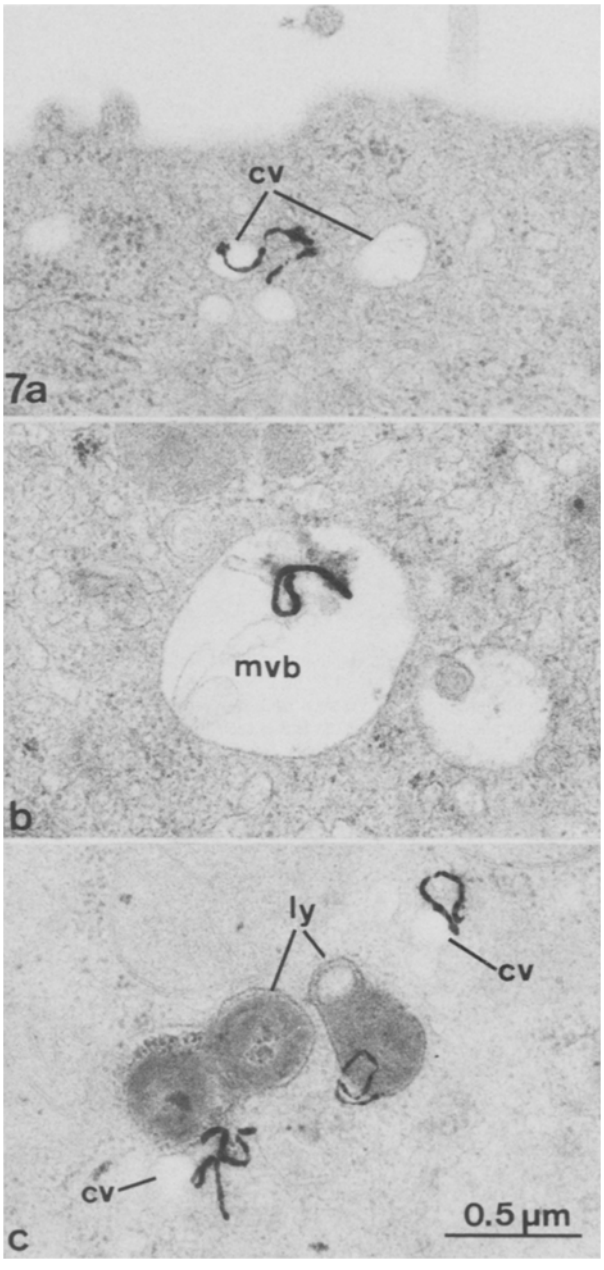

Figure 7. Thin section of isolated rat hepatocytes incubated for 60 min at 37 °C with ^{125}I-insulin. Autoradiographic grains are seen overlying or in the vicinity of clear vesicles (a and c), multivesicular bodies (b) and dense bodies (c). (cv, clear vesicles; mvb, multivesicular bodies; ly, lysosomes). × 36,000. Reproduced from ref. 75 with permission.

Later steps of endocytosis (steps 6, 7 and 8)

The coated vesicle, as mentioned, has a short half-life and the next structure with which the labeled ligand associates is a larger clear vesicle or endosome[32] (fig. 7). These structures have been shown to have important functional properties. Maxfield and associates have shown that the endosome has a proton pump in its membrane capable of acidifying the internal milieu of the vesicle[84]. This acidic environment has important functional consequences for many different types of ligands: for viruses, acidification allows the viral membrane to fuse with the vesicle membrane so that the virus can penetrate the vesicular membrane and gain access to the cytoplasm[64]; for the transferrin system, acidification allows diferric iron to separate from its carrier protein and gain access to the cell[62]; for many other ligands, including the polypeptide hormones, acidification promotes dissociation of ligand from receptor, thus allowing the ligand to be sequestered and processed separately from the receptor.
The next structure seen is the multivesicular body (fig. 7). This organelle is larger than the clear endosome and is further characterized by occasional or many small vesicles. The biogenesis of these structures is unclear; whether they exist de novo or are formed by the fusion of endosomes and other small vesicles remains to be established. The multivesicular body appears to be an intermediate in a continuous process; the next structure visualized is, therefore, the lysosome[32] (fig. 7). Originally the lysosome

was thought to be unique in two ways: a) by containing a proton pump capable of rendering its internal environment acidic and b) by containing biologically active enzymes with an acidic pH optimum. This structure has, therefore, lost some of its novelty since the internal environment of both the endosome and multivesicular body are also acidic. Though the lysosome has distinctive morphologic features, strictly speaking it can be identified only by cytochemical techniques that reveal biologically active enzymes. These techniques may be relatively insensitive unless rigorously applied or multiple enzymes are sought. Thus, it is most appropriate to call structures like multivesicular bodies which have been considered a form of lysosome as 'lysosome-like.' Finally, ambiguity of nomenclature is avoided if one considers the endosome, multivesicular body and lysosome as a continuum involved in processing of ligands, their receptors or both.

Receptor recycling

The concept of recycling of plasma membrane components was first elucidated on kinetic grounds. Steinman and associates observed that the rapid turnover rate of the plasma membrane of L-cells required the de novo recycling of membrane components and was more rapid than could be explained by new protein synthesis[80,82]. It was further shown, using a novel membrane iodination technique, that internal membranes such as lysosomal membranes could cycle to the plasma membrane[67]. Other studies indicated that exocytosis was coupled to endocytosis in such a way as to maintain cell shape and constancy of surface area[71].

For the polypeptide hormones such as insulin it has been shown both biochemically[50,65] and morphologically[35,45] that the receptor is internalized. It has further been shown morphologically that the receptor may be recycled to the plasma membrane[12,36]. Thus, the receptor may be recycled, degraded or both situations may apply. The fate of the receptor following internalization may be the same or different from the ligand. That is, an uncoupling step may take place following initial endocytosis. The nature of this uncoupling step is obscure with respect to the polypeptide hormones.

In other systems, however, where large numbers of receptors are available for analysis, it has been shown that specific structures participate in the uncoupling step. For instance, when the asialoglycoprotein receptor and ligand were separately revealed using immunocytochemical techniques that involved colloidal gold particles of different size, separation of the two components was visualized morphologically[39]. The ligand appeared destined for the lysosome, whereas the receptor entered a tubulovesicular compartment (CURL) thought to be involved in returning the receptor to the plasma membrane. Several features of the uncoupling process appear to be important. The early endocytotic vesicles has an acidic pH (pH $\sim$ 5.4) which promotes separation of the ligand and receptor[84]. It is well known that insulin is rapidly dissociated from its receptor under acidic conditions. While separation of ligand and receptor is not required for recycling, since insulin covalently bound to its receptor will recycle, it is likely that rapid recycling requires this separation. Thus, the acidic endosome is likely the first step in recycling. Morphologically speaking covalently bound insulin receptor complexes recycle in the same types of vesicles as described for endocytosis per se[12].

In other model systems it has recently been shown that an actively recycling receptor such as the transferrin receptor is further segregated into a compartment with a pH of 6.5, i.e., mildly acidic structures, whereas α-2 macroglobulin under similar conditions is transferred to a vesicle of pH 5.4[90]. It is further known that agents that inhibit acidification of endosomes, lysosomes and related structures also inhibit recycling[5,10].

The precise morphologic steps involved in the recycling of the insulin receptor are unknown and we tentatively illustrate this as a continuous process of membrane retrieval (fig. 8). Whether the recycled vesicle enters the insertion pathway of de novo receptor synthesis is also unknown.

What determines the specificity of receptor mediated endocytosis?

We have indicated the general traffic pattern involved in the receptor mediated endocytosis of a polypeptide hormone. The structures involved appear to be common for a wide variety of ligands. For instance, cationic ferritin, a ligand that binds simply to anionic sites on the plasma membrane, is endocytosed by the same general structures as is insulin[31,32,34]. Further, we have shown that LDL and epidermal growth factor (EGF), two ligands that bind to different receptors, are taken up not only by the same type of structure, but by exactly the same structures. Thus, EGF and LDL co-localize to the same endosomes and lysosome-like structures during the process of endocytosis[14].

Thus, specificity lies in the receptor per se. The subsequent anatomical compartments are relatively non-specific. Another kind of specificity must reside at the level of recycling since receptors in the apparent same compartments are recycled at very different rates.

'Apparent' areas of controversy involving the intracellular localization of polypeptide hormones

In addressing areas of apparent controversy, it is important to emphasize that we are expressing a point of view not necessarily a resolution of the controversy.

Up until this point we have emphasized the coated vesicle, endosome and lysosome-like structures in the intracellular movement of endocytosed ligands and their respective receptors (fig. 1). Other workers, however, have emphasized the nucleus and the 'Golgi' as sites of intracellular localization.

Goldfine et al. have emphasized a direct nuclear action of insulin. The main point of concern here is whether ^{125}I-insulin can be localized to the nucleus of the cell morphologically. Two cell types have been studied, the IM-9 cultured human lymphocyte and the freshly isolated rat hepatocyte or perfused liver. We consistently find that the IM-9 cell internalizes insulin but to a very small

extent[13,46], whereas Goldfine et al. find more extensive internalization[41]. Since the methods of analysis are similar, the reasons for these differences are unclear. Using a photoreactive insulin, Olefsky also finds a very low internalization rate in IM-9 cells[7]. While all agree that ^{125}I-insulin is not localized to the nucleus per se in either hepatocytes or IM-9 cells[17,40,41,46], Goldfine et al. find localization to the nuclear membrane of these cells[40,41] and we do not. Thus, the controversy here is technical.

Posner, Bergeron and their associates have emphasized the initial localization of ^{125}I-insulin to the 'Golgi'[6,59]. 'Golgi' localization has been based on two types of studies. When ^{125}I-insulin was injected into the rat and allowed to circulate for a few minutes, and the liver tissue removed from the sacrificed rat was homogenized and fractionated by isopycnic techniques, it was shown that labeled insulin co-migrates with 'Golgi' marker enzymes. The second type of study was morphologic where it has been suggested that labeled insulin localizes with high frequency to lipoprotein filled vesicles. These vesicles are considered to be 'Golgi' related.

In our initial studies using EM autoradiography in both hepatocytes and lymphocytes we found labeled insulin preferentially localized to lysosome-like structures. Though we indicated that some form of endocytotic vesicle must be interposed, we were unable to specifically identify these structures due to their small size[13]. Using 3T3-L1 adipocytes, however, an endosomal structure could be clearly identified[32]. More recently, in experiments employing colchicine which slows the passage of ligand from the plasma membrane to the lysosome, we have clearly identified an endosomal compartment in rat hepatocytes[75]. Further, neither we nor others have ever localized labeled insulin to the 'Golgi' cisternae[19,32]. Thus, the issue is whether the ligand is localized in lipoprotein-rich vesicles distal to the 'trans' Golgi cisternae. These vesicles, however, may be either secretory or endocytotic and are not specific for Golgi[25]. It is possible that more specific approaches will be able to distinguish the secretory from endocytotic nature of these vesicles[52].

Thus, we see this controversy as being in part semantic. In our view, labeled insulin is initially internalized into an endosomal compartment. These endosomes appear to have similar but not identical isopycnic properties to the structures containing 'Golgi' markers and, therefore, the term 'Golgi' fractions does not seem appropriate to describe these vesicular structures.

Biochemical features of receptor mediated endocytosis

The plasma membrane of cells turns over by endocytosis on a continuous basis. Macromolecules, therefore, are continuously taken up by adsorptive or receptor mediated endocytosis. Since binding concentrates the ligand, adsorptive endocytosis is a much more efficient process. Adsorptive endocytosis may proceed continuously, be ligand induced or both. For instance, agents that inhibit receptor recycling may augment cell surface receptor loss for LDL[5]. This suggests that, at least in part, receptors are internalized and recycled on a continuous basis. On the other hand, recycling inhibitors do not alter the insulin receptor over short periods of incubation unless the ligand is present[10]. This suggests that endocytosis is ligand induced.

The mechanism by which the ligand induces endocytosis, however, is unknown. It was initially suggested on the basis of inhibitor studies that transglutaminases were involved[28]. This thesis was based on the idea that several chemical agents inhibited both transglutaminase activity and endocytosis. It is now clear, however, that the various amines and other agents that may affect enzymatic activity of transglutaminases do not inhibit endocytosis[47,60].

More recently it has been shown that the receptors for EGF[26], insulin[58] and PDGF[30] undergo a specific ligand-induced tyrosine-specific autophosphorylation. Since these three ligands and their receptors are taken up by cells in a similar way[16,42,69], it is tempting to speculate that receptor phosphorylation might represent a general mechanism that triggers endocytosis. This would be consistent with the idea that this is an energy consuming process. Recently it has been suggested that phosphorylation may be an important mechanism by which the endocytosis of the transferrin receptor is triggered[61,66]. This is based on phorbol ester induced phosphorylation of the receptor and in this case serine phosphorylation is implicated.

We have recently carried out a series of experiments to test the hypothesis that phosphorylation might be a general mechanism involved in receptor mediated endocytosis of polypeptide hormones. Insulin is internalized by IM-9 lymphocytes, and its receptor phosphorylation is also triggered by insulin binding. We reasoned that if phosphorylation were a general mechanism, the growth hormone receptor of these cells should also be phosphorylated. However, using a variety of conditions and the insulin receptor as a positive control, we have been unable to demonstrate autophosphorylation of the growth hormone receptor[2]. Finally, we have shown that an autoantibody directed against the insulin receptor and manifesting insulinomimetic properties is internalized by cells in an analogous fashion to insulin[24,57]. This autoantibody does not stimulate receptor autophosphorylation[92]. Thus, our current concept is that phosphorylation is not the general mechanism triggering polypeptide hormone endocytosis.

Functional implications of receptor mediated endocytosis

The functional implications of receptor mediated endocytosis depend totally on the ligand. For instance, the LDL receptor mediates the internalization of the cholesterol-containing lipoprotein; cholesterol is liberated by lysosomal enzyme activity and, in turn, the free cholesterol regulates several key enzymes as well as the LDL receptor. Viruses gain entry into the cell and the acid milieu of the endosome allows for fusion of the viral membrane with the vesicle membrane resulting in escape of the virus into the cytoplasm. For transferrin, the endocytotic process regulates the delivery of iron to the cell. For the polypeptide hormones and growth factors, however, the main functional consequences of receptor mediated endocytosis appear to be hormone degradation and receptor regulation.

Different polypeptide hormones transduce an intracellular signal in different ways following their binding to cell surface receptors. Some polypeptide hormones, such as glucagon, activate adenylate cyclase by a complex coupling mechanism. Others, such as insulin, EGF and other growth factors work through an as yet obscure mechanism possibly in part involving receptor autophosphorylation. Yet all of these and other polypeptide hormones undergo a very similar pattern of endocytosis. It is possible that intracellular transport of phosphoproteins subserves some function, but the nature of this putative function is unknown. Thus, the possible role of endocytosis in hormone action, if any, is obscure.

If cell surface binding activates hormone action, then there must be a regulatory mechanism to terminate that action. For instance, insulin degradation has been shown to be receptor linked[83]. Thus, internalization may be an important mechanism for removing the hormone from the cell surface and initiating its degradation. For certain polypeptides such as EGF and growth hormone, it is likely that ligand degradation is primarily lysosomal. This is supported by the lysosomal localization of these peptides and inhibition of their degradation by agents that alkalinize the endosome and lysosome. A similar process is true for insulin in that insulin is internalized into exactly the same compartments as are the other hormones, and insulin degradation is also inhibited by so called lysosomotropic agents such as chloroquine, ammonium chloride and other similar compounds[47]. In the case of insulin, however, neutral proteases also have been implicated in its degradation[47]. While there is no question that soluble neutral proteases will rapidly degrade insulin, it is not totally clear that these proteases have access to the hormone in the intact cell. It must be remembered that the early endocytotic vesicles are also acidic, making it unlikely that these neutral proteases are active during the early phases of endocytosis.

Our current thinking is that insulin degradation occurs in part through a lysosomal mechanism but that neutral proteases may also play a role. Whether these proteases act solely on the cell surface, however, remains to be determined.

Another general feature or consequence of hormone binding to receptor is ligand induced receptor regulation[38]. Since both the hormone and receptor are internalized it is clear that endocytosis is an important mechanism of modulating cell surface receptor concentration. Further, it is clear that there may be no simple relationship between ligand and receptor internalization and receptor down regulation.

This is true because several regulatory events are involved. Growth hormone in cultured lymphocytes, and EGF binding in human fibroblasts, are two relatively straightforward examples. For instance, small concentrations of hormone induced receptor down regulation, and receptor down regulation and ligand internalization, are highly correlated as a function of time[4, 9, 42, 54]. In the case of growth hormone the ligand is, in part, irreversibly bound, and down regulation is further enhanced by ammonium chloride[4]. Thus, in this system there appears to be little recycling and what recycling does occur is inhibited by alkanization and inhibition of ligand dissociation.

The lack of a straightforward relationship between down regulation and internalization relates to recycling. We have pointed out that at some step in the endocytotic pathway the ligand and the receptor may become uncoupled and each may have a different fate. Thus, the receptor may be recycled, may be degraded, or some combination of these events may take place. In the U-937 human monocyte cell line, small concentrations of insulin induce the loss of cell surface receptors. These receptors are in part recovered in soluble extracts of the cell. Thus, in this case, receptors appear to be internalized and in part recovered intact intracellularly and in part are degraded. Monensin, which inhibits recycling, augments down regulation. Monensin, however, does not affect that fraction of receptors destined for degradation. Thus, monensin affects only the recycling component of the receptor. In other cell types where the rate of internalization is low, such as in the IM-9 lymphocytes, monensin has essentially no effect[10].

Hormone and receptor internalization also provide a mechanism for understanding receptor regulation in clinical states. Thus, nontarget cells like peripheral blood monocytes have insulin receptors. The monocyte insulin receptor is regulated in an analogous fashion to hepatocyte receptors. Monocytes and hepatocytes exposed to the same concentration of insulin would, therefore, regulate their receptors in an analogous fashion by way of receptor mediated endocytosis[48].

Further evidence that receptor mediated endocytosis is a regulated process comes from studies in hypoinsulinemic states. If it is true that endocytosis is a mechanism to remove the ligand from the cell surface and terminate its signal, it might be expected that in hypoinsulinemic states that this process would be impaired. We have recently found that in streptozotocin induced diabetes in the rat, a hypoinsulinemic state, the internalization of ^{125}I-insulin is impaired. By contrast, the internalization of ^{125}I-glucagon is either normal or increased[22]. Further in insulinopenic type I diabetes of man, ^{125}I-insulin internalization is markedly inhibited[21].

Conclusion

The insulin receptor is an integral protein of the plasma membrane of the cell. It is composed of two subunits: an α subunit, which binds the hormone, and a β subunit which is a tyrosine specific protein kinase capable of undergoing autophosphorylation. These independent subunits are synthesized by way of a higher molecular weight single chain precursor and thus are the product of a single gene[29, 49, 85] localized to chromosome 19[29, 91]. Assuming that the insulin receptor is synthesized in the same fashion as other integral membrane glycoproteins, then the nucleus, the rough endoplasmic reticulum, and the Golgi apparatus are involved in its biosynthesis. Further, there must be some form of transport of the mature receptor subunits to the plasma membrane where they are inserted.

By contrast, the endocytotic route involves coated pits, coated vesicles, large clear vesicles or endosomes, multivesicular bodies and other lysosomal forms. In addition, it is possible that some other as yet unidentified organelle

is involved in recycling (fig. 8). At the present time, with respect to the insulin receptor, the biosynthetic pathway and the endocytotic pathway appear to be separate. Further, it does not appear that either pathway, i.e. synthesis or endocytosis, exerts a regulatory function over the other.

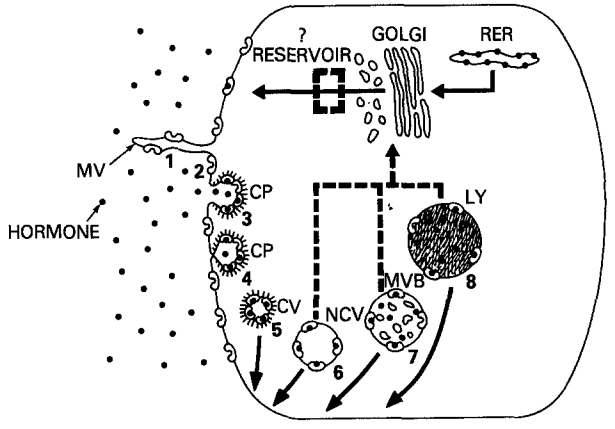

Figure 8. Synthesis and retrieval of polypeptide hormone receptors. Steps 1–8 are the same as shown in figure 1. The solid arrows represent points at which membrane receptors can recycle back to the plasma membrane. The dashed arrow indicates that recycling could also occur by way of the synthetic pathway for membrane integral proteins. A potential reservoir is shown to indicate, under some circumstances, there may be a preformed pool of intracellular receptors. Reproduced from ref. 43 with permission.

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The nature and development of steroid hormone receptors*

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Models of steroid hormone action

Our current conceptualization of the primary steps in steroid hormone (estrogens, androgens, progestins and corticoids) action is shown in figure 1[10]. We now believe that the steroids, which are generally hydrophobic molecules, diffuse through the outer cell membrane and cytoplasm to the nucleus[47]. In the nucleus the steroids bind to their respective receptors which are assumed to be bound with low affinity to some nuclear component[35]. This nuclear component could be DNA, nuclear matrix or some chromatin protein (acceptor) but this has not been proven conclusively. As a result of the steroid-receptor interaction, the receptor's conformation changes dramatically and its affinity for nuclear components becomes much higher[11, 13, 51]. This change in the association of receptor with nuclear components is correlated with, and may lead to the rapid (within minutes) changes in gene expression observed in the target tissues[1, 25, 29]. This general model may apply to all the steroid hormones, vitamin D metabolites and thyroxine, but has not been clearly demonstrated for all such hormones.

The model described above has undergone considerable revision in the last few years. Studies in the 1960s had shown that unoccupied steroid receptors were readily solubilized in cytosolic tissue extracts prepared by conventional homogenization procedures[41]. In contrast, the occupied (steroid bound) receptors were not readily solubilized and were bound in high proportion to nuclear fractions[39]. This led to the proposition of the translocation or two-step model of steroid hormone action[12, 16]. In the translocation model the receptor is initially a cytoplasmic protein. Upon binding steroid the receptor undergoes conformational changes which result in the steroid-receptor complex translocating to the nucleus where it binds to its site or sites of action. Early auto-