Of course whistle-blowers are not always right. They might be motivated by personal malice, they may be cranks, or they may be honest, but mistaken. Both common sense, and a sense of loyalty to the employer, dictate an earnest effort to settle differences of opinion by working within the organization. However, if higher authorities fail to respond, and if the matter appears to involve serious issues of human safety and health, it may be necessary to bring the matter to public attention. The individual who takes such a risk obviously needs good legal advice and other kinds of help (*15*). Our complex society needs increasing input from those who perceive otherwise unnoted risks or opportunities and bring messages that may be unwelcome to established authorities. To use criticism and dissent constructively in dealing with both risks and opportunities, clear policies are needed, with definitions of procedures for due process in controversial cases and, if necessary, formal hearings and a possibility of appeal.

The polarization of opinions on some issues today is disturbing. The conflict between the advocates and enemies of nuclear power is one example; the dispute over the origins of cancer is becoming another. Richard Peto (*16*) described the distortions and untruths promoted by tobacco companies in their efforts to discredit the overwhelming evidence for the relation between smoking and lung cancer. At the same time he severely criticized some of the alleged evidence that would ascribe nearly all cancers to toxic substances introduced by man. S. S. Epstein, whom Peto sharply criticized, has responded vigorously (*17*). The gravity of the hazard from industrial carcinogens, to workers and others, is clear; but their relative role in the totality of human cancers is still hotly debated. In the bitterness of such controversies, either side may distort data. As Peto remarked, "Scientists on both sides of the environmentalist debate now have career interests at stake." But it is important above all that the passion for getting at the truth should be the dominant passion for scientific workers when they are trying to act as responsible scientists. That may appear sometimes to be an unattainable goal in the atmosphere of current debate, but it is worth striving for, both to maintain the confidence of the public and to keep confidence in ourselves.

References and Notes

1. A. Weinberg, *Minerva* **16**, 1 (1978).
2. R. K. Merton, in *The Sociology of Science* (Univ. of Chicago Press, Chicago, 1973), pp. 267-278; A. Cournaud and M. Meyer, *Minerva* **14**, 79 (1976).
3. J. Monod, in *Collected Works of Leo Szilard: Scientific Papers*, B. T. Feld and G. W. Szilard, Eds. (MIT Press, Cambridge, Mass, 1972), p. xvi.
4. S. Andreopoulos, *N. Engl. J. Med.* **302**, 743 (1980).
5. R. Carson, *Silent Spring* (Houghton Mifflin, Boston, 1962).
6. U.S. President's Science Advisory Committee, "Use of pesticides" (The White House, Washington, D.C., 1963); the report was reprinted in *Chem. Eng. News* **41** (No. 21), 102 (1963). J. Primack and F. von Hippel [*Advice and Dissent: Scientists in the Political Arena* (Basic Books, New York, 1974), pp. 41-46] provide a useful account of the effect of the report.
7. Federation of American Scientists, "To whom are public interest scientists responsible" (Public Interest Report 29, Washington, D.C., 1976).
8. C. Djerassi, *Science* **169**, 941 (1970); *Bull Am. Acad. Arts Sci.* **32**, 22 (1978).
9. E. Ashby, *Perspect. Biol. Med.* **23**, 7 (1979).
10. R. Chalk and F. von Hippel, *Technol. Rev.* **81**, 48 (1979).
11. J. T. Edsall, *Scientific Freedom and Responsibility* (AAAS, Washington, D.C., 1975).
12. L. J. Carter, *Science* **207**, 1057 (1980).
13. C. Holden, *ibid.* **210**, 749 (1980); J. L. Lawler, *ibid.* **211**, 875 (1981).
14. D. W. Ewing, *Freedom Inside the Organization: Bringing Civil Liberties to the Workplace* (Dutton, New York, 1977). See also A. F. Westin, Ed., *Whistle Blowing! Loyalty and Dissent in the Corporation* (McGraw-Hill, New York, 1981).
15. P. Raven-Hansen, *Technol. Rev.* **82**, 34 (1980).
16. R. Peto, *Nature (London)* **284**, 297 (1980).
17. S. S. Epstein, *Nature (London)* **289**, 115 (1981); _____ and J. B. Swartz, *ibid.*, p. 127. Compare J. Cairns, *ibid.*, p. 353.

18. I am much indebted to my fellow members of the AAAS Committee on Scientific Freedom and Responsibility for our continued joint work relating to many of the problems discussed in this article. I am particularly indebted to R. Baum, F. von Hippel, J. Primack, and R. Chalk. This article is, however, an expression of my personal views. This work was supported by grant SOC7912543 from the National Science Foundation.

Polypeptide-Binding Membrane Receptors: Analysis and Classification

Jerry Kaplan

Mammalian plasma membranes are in general impermeable to molecules of high molecular weight. Thus mechanisms are present by which the structural elements, or information content, of macromolecules are made available to cells. Fluid-phase pinocytosis is one mechanism by which macromolecules may gain access to intracellular compartments. However, fluid-phase pinocytosis is nonselective; that is, uptake of molecules is solely a linear function of their extracellular concentration (*1*). In addition, endocytosis is probably a mechanism for termination rather than initiation of information transfer. To overcome the lack of selectivity of fluid-phase endocytosis, cells have developed plasma membrane receptors capable of forming high-affinity complexes with specific ligands.

There is great diversity in the chemical nature of both ligands and the receptors to which they bind. However, studies in my laboratory and elsewhere suggest that membrane receptors for soluble polypeptides may be divided into two categories. The basic distinction between receptors is that the binding of ligand by class I receptors leads to changes in cell behavior or metabolism. These changes result from the interaction of ligand and receptor at the cell surface and, although ligand internalization may occur, it is not a prerequisite for ligand function. The major role of class II receptors is to mediate ligand internalization. Binding of ligand to class II receptors does not per se lead to alteration of cell activity. Modifications of cell behavior, if they occur at all, are consequences of ligand metabolism. Receptors in each class show similarities in their divalent ion requirements, topographical distribution, and regulation.

In this article I consider only receptors for soluble polypeptides, excluding those for cholera and diphtheria toxin. A major caveat to any analysis of receptor behavior is that, with rare exceptions, what is measured is not receptor molecules but

The author is an associate professor in the Department of Pathology, University of Utah Medical School, Salt Lake City 84132. The article was written when Dr. Kaplan was an assistant professor in the Department of Physiology, University of Connecticut Health Center, Farmington.

0036-8075/81/0403-0014$01.75/0

Summary. Polypeptide receptors on mammalian plasma membranes can be categorized on the basis of function. The binding of ligand by class I receptors results in changes in cell metabolism or behavior. Hormone-receptor interactions typify this group. The binding of ligand by class II receptors results in ligand internalization. Although changes in cellular activity may result from metabolism of the internalized ligand, the interaction between ligand and class II receptor does not itself lead to alterations in cell behavior. Class II receptors include those for low-density lipoproteins and for α-macroglobulin–protease complexes. Although receptors within each category are chemically disparate, they show striking similarities in behavior. Analysis of the behavioral patterns of receptors in each category reveals insights into receptor physiology and allows for a prospective analysis of receptor characteristics.

receptor function; that is, ligand binding. Because in many instances the stoichiometry of ligand-receptor interaction is unknown, knowledge of receptor properties is at best qualitative. For example, the quantitative definition of receptors is unclear in systems where receptors may be clustered and thus ligand binding may be multivalent. In such systems determination of receptor distribution or turnover may be subject to error. Despite these caveats, analysis of receptor behavior may lead to insights in receptor function.

Class I Receptor

The categorization of membrane receptors is based on whether the major function of the receptor is to transmit information (class I receptors) or internalize ligand (class II receptors). All of the receptors in class I, after interaction with ligand, impart information that subsequently modifies some aspect of cell behavior or metabolism. Receptors in class IA are the traditional hormone receptors, whereas those in class IB are receptors for other, humoral nonhormonal agents. The immunoglobulin E (IgE) receptor of the mast cell is a prototype of the latter group (*2*). The distinguishing feature of these receptors is that binding of agents to receptors leads to modulation of metabolic activity without requiring internalization of ligand or receptor. Studies indicate that molecules other than ligand which interact with receptors can also effect information transfer. For example, antibodies to the insulin (*3*) or IgE receptor (*4*) mimic the specific ligand. Such observations suggest that the information required to modify cell behavior is encoded in the receptor, and that ligand presumably alters receptor conformation and triggers a cellular response. Because there is already extensive literature on hormone receptors, the emphasis in this article will be on type II or scavenger receptors.

Class II Receptor

The distinguishing feature of class II receptors is that their major, if not sole, function is to mediate ligand internalization and thus remove the ligand from the extracellular environment (Table 1). A long-term inability to internalize ligand, or an excessive internalization of ligand, may lead to pathophysiological sequelae. However, occupancy per se of class II receptors by ligand does not appear to lead to any alteration in cell behavior or overall metabolic activity. Two examples illustrate this point: human fibroblasts incubated in vitro exhibit identical rates of macromolecular synthesis and growth in the presence or absence of low-density lipoprotein (LDL) (*5*); cells incubated in the presence or absence of transcobalamin II–cobalamin complexes also exhibit similar growth rates (*6*). Because of the high intracellular concentration of cobalamin, prolonged cobalamin deprivation would be required before any effect on cell growth would be seen (*7*). Thus, aside from events resulting from the metabolism of internalized ligand, the act of binding and internalization of ligand does not appear to convey any signal or information.

Ligand internalization by class II receptors provides the cell with a required factor, for example, cobalamin (*6*), or a substrate such as cholesterol, which in energetic terms may be more expensive to synthesize than import (*5*). Class II receptors also internalize, and thus remove from biological fluids, potentially noxious agents, of which both α-macroglobulin–protease complexes (*7*) and mannose-terminal glycoproteins (*8*) are prime examples. In some instances internalized ligand may be transferred out of the cell rather than stored or catabolized. Thus class II receptors can mediate transcellular movement of macromolecules. An example of this is the uptake of maternal immunoglobulin G (IgG) by yolk sac receptors, providing passive immunity to the embryo (*9*).

Distinctions Between Class I and Class II Receptors

Divalent ion requirement. Although both classes of receptors exhibit high-affinity binding with their specific ligand, binding of ligand to class II receptors is dependent on divalent cations, whereas binding of ligand to class I receptors is divalent cation independent (Table 2). For example, there is no measurable rate of dissociation of α-macroglobulin–[^{125}I]trypsin complexes from macrophage surface receptors when cells are incubated in Ca^{2+} containing buffers. Removal of Ca^{2+} by addition of the chelating agent EDTA promotes immediate dissociation of ligand-receptor complexes (*10*). Binding of asialoglycoproteins to the purified hepatic binding protein is also Ca^{2+} dependent, and ligand-receptor complexes dissociate in the presence of chelating agents (*11*). Similarly, binding of ^{125}I-labeled LDL's to membrane fragments is Ca^{2+} dependent (*12*).

Dissociation of receptor-ligand complex. Hormone (H) or class I receptor (R)–ligand interactions are conventionally treated as reversible reactions

$$H + R \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} HR$$

where the dissociation constant K_D is defined as K_{-1}/K_1. The dissociation of ligand from receptors is measurable and may represent a physiological mechanism by which hormone-mediated information transfer may be regulated. Thus when the concentration of extracellular hormone is lower than K_D, dissociation of hormone-receptor complexes will occur. α-Macroglobulin–trypsin complexes (*10*), or asialoglycoproteins (*11*) bound to receptors on cells or membrane fragments, do not exhibit measurable rates of dissociation. When there is no significant K_{-1} (or "off rate"), analysis of (class II) receptor ligand interactions in terms of K_D is inappropriate. Accordingly, standard analyses of ligand receptor interaction (that is, Scatchard analyses) are precluded. However, not all class II–ligand interactions are typified by exceedingly slow rates of receptor-ligand dissociation. Under some conditions, binding of ^{125}I-labeled IgG to yolk sac membranes appears to be reversible (*13*).

Clearly, time is implicit in the term "measurable rate of dissociation." Receptor-bound LDL (*14*) or α-macroglobulin–protease complexes (*15*) are internalized within minutes; thus under physiological conditions measurement of

rates of dissociation of ligand-receptor complexes may be precluded. What appears to be important is not the rate of dissociation but the rate of dissociation relative to the rate of internalization.

Internalization of Ligand and Regulation of Class II Receptors

That the major function of class II receptors is internalization is demonstrated by the finding that these receptors may be reutilized. After the binding of a ligand to surface receptors, the ligand is internalized and either accumulates in a subcellular compartment or is passed through the cell, while the number of surface receptors remains unchanged. Analysis of the kinetics of ligand uptake indicates that the same complement of surface receptors is capable of entering into further cycles of ligand binding and internalization. Most of the observations on reutilization have been made during studies of the fibroblast LDL receptor (*14*), the macrophage α-macroglobulin–protease complex receptor (*16*), and the macrophage receptor for mannose terminal glycoconjugates (*9*).

Receptor-mediated endocytosis is extremely rapid; the half-life of ligand bound to either the fibroblast LDL receptor (*14*) or the macrophage α-macroglobulin–protease receptor (*15*) is 2 to 4 minutes. The internalization of receptor-bound α-macroglobulin–protease complex can be described by first-order kinetics, suggesting that internalization of ligand is a random process and that most (≃ 95 percent) receptor-bound ligand has an equal probability of being internalized (*15*).

Experiments show that:

1) Cells are capable of internalizing, in the steady state, an amount of ligand in excess (greater than five- to tenfold) of the amount of ligand bound to surface receptors.

2) The rate of ligand uptake cannot be ascribed to receptor biosynthesis de novo, because the rate of receptor synthesis, K_S, is about 1 percent per hour for the LDL receptor (*17*) and 15 percent per hour for the α-macroglobulin-protease complex receptor (*18*), which is much less than the rate of ligand uptake.

3) Direct examination of fibroblasts (*12*) and macrophages (*16*) has produced no evidence of large internal pools of receptors, indicating that replacement of internalized ligand receptor complexes does not occur by insertion of receptors from some reservoir.

Thus, although the internalized ligand is catabolized to its components, the surface receptors are spared from catabolism and reutilized. Support for this idea is provided by other observations. Uptake of asialoglycoproteins by rat hepatocytes does not affect either receptor number or half-life (*17*). This result implies that the catabolism of internalized asialoglycoproteins is not accompanied by catabolism of receptors. However, only 10 percent of the parenchymal cells' content of receptors are expressed on the surface, the remainder being localized on intracellular membranes. Thus the possibility remains that ligand uptake reflects the continued recruitment of receptors from an intracellular reservoir. Doyle *et al.* recently fused hepatocyte plasma membrane vesicles to mouse L cells (*19*). Mouse L cells do not have receptors for asialoglycoproteins and cannot bind these glycoproteins. After fusion with hepatocyte membrane vesicles, the mouse L cells could mediate several rounds of ligand uptake. This experiment indicates that continued ligand uptake did not result from recruitment of receptors from an intracellular reservoir. Rather, ligand uptake resulted from the reutilization of the complement of surface receptors. Further support for this view comes from the study of Stockert *et al.* (*20*) who demonstrated that perfusion of rat liver with antibody to receptor led to sustained and specific inhibition of ligand uptake. Thus, although hepatocytes contain intracellular asialoglycoprotein receptors, they do not appear to play a role in the internalization of extracellular ligand.

Fetal yolk sacs have the ability to accumulate selectively large quantities of phosphovitellogen, IgG, and albumin (*9*). The amount of material accumulated is such that it is energetically unfeasible to synthesize new receptors for each molecule of ligand internalized. While this line of reasoning is at best suggestive, it is consistent with the view that receptors may be reutilized.

In contrast to class II receptors, evidence suggests that class I receptors may be utilized once or, at best, only a limited number of times. Thus, binding of ligand may be followed by the internalization and catabolism of receptor-ligand complexes. This phenomenon has been studied with the use of epidermal growth factor (*21*), human chorionic gonadotropin (*22*), and insulin (*23*). Binding of ligand leads to the internalization of ligand and the disappearance of a significant fraction of surface receptors. Reappearance of receptor activity requires protein synthesis and is blocked by cycloheximide. These observations indicate, but do not prove, that receptor reappearance results from de novo receptor biosynthesis.

The rates at which ligand-receptor complexes are internalized vary widely: 90 percent of cell-bound epidermal

Table 1. Ligands that bind to class II receptors. The major function of class II receptor is ligand internalization (N.D., not determined).

Ligand	Function of receptor-ligand complex	Regulation of receptor number or activity by ligand	Topological distribution	Dependence of ligand-receptor binding on divalent ions
Low-density lipoproteins	Supplies cholesterol	Receptor synthesis inhibited by cholesterol (*18*)	Pits (*59*)	+(*12*)
α-Macroglobulin–protease	Removes injurious agents	No (*16, 30*)	N.D.	+(*10*)
Glucose or mannose terminal glycoproteins	Removes injurious agents	No (*8, 10*)	N.D.	+ (*8*)
Galactose terminal glycoproteins	Removes injurious agents	No (*17*)	N.D.	+ (*11*)
IgG yolk sac receptor	Fetal immunity	No	(Pits)* (*9*)	+ (*13*)
Phosphovitellogen	Supplies source of protein for embryo	No	(Pits)* (*9*)	+ (*13*)
Fibrin	Removes injurious agents	N.D.	N.D.	+ (*34*)
Cobalamin–cobalamin-II	Supplies vitamin B_{12}	No (*29*)	N.D.	+(*29*)

*Evidence inconclusive.

growth factor was internalized with a half-time ($T_{1/2}$) of 2 minutes at 37°C (*21*), whereas in 30 minutes only 50 percent of hepatic parenchymal cell insulin receptors were internalized (*24*). Factors that control the rate of internalization of class I receptor–ligand complexes have yet to be defined. Internalization of ligand-receptor complexes may be a mechanism by which modifications of cell behavior or metabolism initiated as a result of class I receptor–ligand complexes may be limited. Internalization would result not only in a decrease in ligand concentration but also a decrease in receptor concentration. Recently, Mayfield *et al.* (*25*) presented data indicating that inhibition of the ability of cells to internalize epidermal growth factor led to an enhancement of that hormone's activity. This result is consistent with the view that internalization limits the response to hormones.

Regulation of Receptor Number or Activity

On exposure to ligand, class I receptors may be down regulated; that is, the number of surface receptors may be reduced. Thus, binding of ligand to class IA receptors leads to regulation of receptor number. The reduction in receptor number may result from an acceleration in the rate of receptor catabolism where the initial step in this process is a ligand-induced receptor internalization. Thus, formation of a ligand-receptor complex is followed by receptor internalization and a consequent decrease in surface receptor concentration. Most class I receptors exhibit this behavior; however, there are not enough data to determine whether a similar phenomenon occurs with class IB receptors. Polymorphonuclear leukocytes incubated in high concentrations of the chemotactic peptide fMet-Leu-Phe, or in C_5A lose their chemotactic activity (*26*). This desensitization may result from either a loss of receptors or an inability of receptor-ligand complexes to transmit information. For example, in recent studies the chemotactically active derivatives of fMet-Leu-Phe were rapidly internalized (*27*), whereas IgE-induced mast cell degranulation was not followed by internalization of receptor bound IgE (*28*). Regardless of whether class I receptors are down regulated or desensitized, reduction in receptor number or activity results in modulation of information transfer.

In contrast to class I receptors, where ligand binding leads to receptor regulation, binding of ligand by class II receptors does not lead to regulation of receptor number or activity. If regulation of receptor number occurs, it appears to result from a metabolite derived from the internalized ligand. A number of examples demonstrate this point:

1) Youngdahl-Turner *et al.* (*29*) demonstrated that incubation of human fibroblasts with high concentrations of either cobalamin- or transcobalamin II–cobalamin complexes for 24 hours did not lead to a reduction in receptor number. The half-life of the transcobalamin receptor is 8 hours. If regulation occurred at the level of receptor synthesis or degradation, the turnover rate of the receptor would have been such that a decrease in receptor number would, presumably, have been observed.

2) Incubation of alveolar macrophages with high concentrations of α-macroglobulin–protease complexes for up to 12 hours did not alter the number of surface receptors (*30*). Receptor half-life was 4 hours (*16*); therefore, if regulation of receptor number occurred by modulation of receptor turnover, this similarly would have been observed.

3) Incubation of alveolar macrophages with high concentrations of mannose terminal glycoproteins did not prevent continued receptor-mediated uptake (*30*), suggesting the continued presence of surface receptors.

4) Injection of galactose terminal glycoproteins into rats did not affect either the number or turnover rate of the galactose terminal glycoprotein receptor of parenchymal cells (*19*).

5) Probably the most well-studied class II receptor is the human fibroblast LDL receptor. Incubation of cells with LDL's will, after a certain time, result in a decrease in receptor number. Conversely, incubation of cells in the absence of LDL's will lead to an increase in surface receptor number. The time course of these changes in receptor number is much greater than the time required for down regulation of class I receptors. Alteration in receptor number results from control of receptor biosynthesis, not degradation (*18*). Additional data suggest that regulation of receptor number results not from the uptake of LDL's per se or the accumulation of LDL's in lysosomes, but from events that occur subsequent to the metabolism of the LDL's. For example, fibroblasts from individuals with Wolman's disease show decreased levels of acid lipase and an inability to hydrolyze cholesterol esters. The fibroblasts exhibit constitutively high levels of LDL receptors and unregulated uptake of LDL's (*31*). Addition of oxygenated cholesterol derivatives to either normal fibroblasts or fibroblasts from patients with Wolman's

Table 2. Ligands that bind to class I receptors. These ligands transmit information as a result of binding to the class I surface receptors (N.D., not determined).

Ligand	Function of receptor-ligand complex	Regulation of receptor number or activity by ligand	Topological distribution	Dependence of ligand-receptor binding on divalent ions
		Ligands (hormones) that bind to class IA receptors		
Chorionic gonadotropic	Regulates hormone production	Down regulated (*22*)	N.D.	– (*22*)
Epidermal growth factor	Metabolic activity or mitosis	Down regulated (*21*)	Randomly distributed (*51*)	– (*21*)
Insulin	Metabolic activity	Down regulated (*23*)	Randomly distributed (*45*)	– (*23*)
Glucagon	Metabolic activity	Down regulated (*62*)	N.D.	– (*63*)
Thyroid-releasing hormone	Hormone release or synthesis	Down regulated (*64*)	N.D.	– (*64*)
		Ligands (humoral agents) that bind to class IB receptors		
IgE (mast cells)	Histamine release	Desensitized (*2*)	Randomly distributed (*4*)	– (*2*)
fMet-Leu-Phe (polymorphonuclear monocytes)	Chemotaxis	Down regulated (*26*)	Randomly distributed (*26*)	– (*26, 65*)
C_5A (polymorphonuclear monocytes)	Chemotaxis	Desensitized (*26*)	N.D.	– (*65*)

disease results in reduction of LDL receptors and, consequently, reduction in LDL uptake (*32*). These observations indicate that regulation of LDL receptors results from regulation of receptor biosynthesis which is controlled by the concentration of free (nonesterified) cholesterol. Thus, regulation of receptor number results from activities "downstream" from ligand binding and internalization.

Unregulated Class II Receptor Activity

Together, these observations suggest that the activity of class II receptors may be unregulated. In those instances where receptors are subject to regulation, the site of regulation appears not to be at the level of ligand binding or internalization. This is understandable in teleological terms in that macrophages, for example, may be exposed to only low concentrations of α-macroglobulin–protease complexes or lysosomal glycosidases for extended periods or to high concentrations intermittently. The high concentrations would result from processes such as acute pancreatitis or, more frequently, lysosomal degranulation of leukocytes. The same argument may be used for the uptake of transcobalamin II–cobalamin complexes; that is, the receptor is exposed to the ligand in low concentrations most of the time, and high concentrations intermittently. Thus, there has been no obvious evolutionary pressure to develop mechanisms of receptor regulation. There appears to be no rationale for regulation of (in terms of decreasing) the accumulation of maternal IgG or phosphovitellogen by the developing yolk sac, since a sustained rate of ligand uptake would be desirable rather than disadvantageous.

Although the reutilization of receptors provide an efficient means of ligand uptake, unregulated uptake can, under certain conditions, lead to pathological consequences. Macrophages respond to a large endocytic load by the de novo synthesis and secretion of neutral proteases among which are elastase, collagenase, and plasminogen activator (*33*). Incubation of alveolar macrophages with high concentrations of α-macroglobulin–protease complexes or mannose-terminal glycoproteins results in collagenase secretion (*30*). Internalization of proteins by fluid-phase pinocytosis will also lead to protease secretion. However, relative to the concentration of α-macroglobulin–protease complexes, a 50- to 100-fold higher concentration (in terms of milligrams per milliliter) of albumin is required to bring about comparable rates of collagenase secretion. These results demonstrate the difference in efficiency of uptake between receptor-mediated and fluid-phase pinocytosis. They also show that uptake of α-macroglobulin–protease complexes, a route for disposal of potentially injurious proteases, may itself induce protease secretion.

Receptor-mediated uptake of other ligands by macrophages may also lead to protease secretion. Mouse peritoneal macrophages exhibit receptors for soluble fibrin–fibrinogen complexes (*34*). Uptake of these complexes provides a mechanism of limiting thrombus formation by preventing the formation of microparticulate fibrin. Data indicate that incubation of mouse peritoneal macrophages with soluble fibrin leads to protease secretion. The concentration of fibrin required to induce protease secretion is higher than that required to saturate surface receptors (*35*). These data suggest that protease secretion may be a consequence of the receptor-mediated uptake of fibrin.

As mentioned above, individuals with Wolman's disease have low concentrations of acid lipase, or none at all, and are unable to metabolize cholesterol esters. These individuals are hypercholesterolemic because of both unregulated cholesterol biosynthesis and unregulated uptake of LDL's.

The initiation or progression of atherosclerosis may also be related to unregulated receptor activity. One of the early signs of an atherosclerotic plaque is the presence of cells containing an excessive intracellular accumulation of cholesterol esters. These "foam cells" can develop in vivo, in animals fed high cholesterol diets (*36*), or in vitro, in cultured smooth muscle cells or macrophages that are exposed to derivitized lipoproteins (*36*). Smooth muscle cells in culture regulate intracellular cholesterol by modulation of either the level of the branch point enzyme in cholesterol biosynthesis, hydroxymethylglutaryl coenzyme A reductase, or the level of LDL receptors. Incubation of LDL's with *N*, *N*-dimethyl-1,3-propanediamine results in the formation of an LDL molecule with a strong positive charge (*37*). This molecule, while it can interact with the smooth muscle LDL receptor, is also taken up by adsorptive endocytosis independent of the normal route of uptake. Uptake by this route is not regulated and exposure of cells to derivitized LDL leads to massive accumulation of intracellular cholesterol esters.

Recently, Goldstein *et al.* (*38*) demonstrated that macrophages were unable to internalize LDL's selectively but had a high affinity for acetylated or maleated LDL's. Binding of acetylated LDL's to cells exhibited all of the characteristics of a receptor-mediated process in that binding was time-dependent, saturable, and exhibited specificity, although the features recognized by the receptor remain to be defined. The rate of uptake of the acetylated LDL by macrophages was unaffected by either cellular cholesterol content or extended prior exposure to acetylated LDL. As a consequence of lack of regulation of receptor function the cells accumulated large amounts of cholesterol esters and developed an appearance similar to that of foam cells in erythematous lesions. These results and the results of Mahley *et al.* (*36*) have led to the hypothesis that environmental factors may result in the generation of altered lipoproteins that may be taken up by receptors not subject to regulation, either of number or activity. Unregulated receptor uptake leading to massive cholesterol ester accumulation may be a casual feature in the development of atheromatous plaques and atherosclerosis (*39*).

Although cells themselves do not regulate uptake of α-macroglobulin–protease complexes, other mechanisms in the body may limit unregulated ligand uptake. For example, cells may not be exposed to high concentrations of α-macroglobulin–protease complexes because proteases introduced into plasma may bind first to other protease inhibitors, specifically to α_1-antiprotease (α_1-antitrypsin). The clearance of α_1-antiprotease–protease complexes is slow and with time proteases are transferred to α_2-macroglobulin (*40*). Thus, in plasma, α_1-antiprotease acts as a reservoir preventing the development of high concentrations of α_2-macroglobulin–protease complexes. Thus unregulated ligand uptake may be prevented or inhibited by noncellular mechanisms (*41*).

Receptor Reutilization and Membrane Recycling

The mechanisms behind reutilization are still unclear. Anderson (*42*) has pointed out that although there is evidence of receptor-mediated ligand internalization, there is no direct evidence of receptor internalization. If such evidence could be obtained, then an association between reutilization and membrane recycling would be established.

The recycling hypothesis has been developed to explain the ability of cells to internalize large quantities of surface membrane yet still retain a constant surface area. Steinman *et al.* (*43*), studying fluid-phase pinocytosis in mouse peritoneal macrophages and fibroblasts (L cells), concluded that these cell types internalized an amount of plasma membrane equivalent to 200 and 25 percent, respectively, of their surface area per hour. The amount of internalized membrane was in vast excess of the biosynthetic capacity of either cell type (*44*). Therefore, it has been hypothesized that in order to maintain a constant surface area, the bulk of internalized membrane must be reinserted back into the plasma membrane or recycled. Similar arguments have been advanced on the basis of studies of pinocytic activity in amoeba (*45*), thyroid epithelium (*46*), and secretory cells (*47*). An analogous conclusion that internalized membrane is recycled is based on a theoretical consideration of the ratios of surface area to volume in pinocytic vesicles and lysosomes (*48*).

However, all the arguments in favor of recycling are based on theoretical considerations or morphometric analyses. If it could be established that during receptor-mediated ligand uptake the receptor is internalized, this would be biochemical evidence of the recycling hypothesis and would provide a theoretical basis for reutilization.

Topographical Distribution of Surface Receptors

It is clear from the preceding discussion that membrane receptors show at least two different patterns of behavior. Certain mechanisms must be able to separate class I receptors, which appear to be irreversibly internalized and degraded following ligand binding, from class I receptors that are reutilized. On the basis of published studies, I suggest that two factors may contribute to the separation process: (i) differences in plasma membrane polypeptides may enable them to be selectively internalized, and (ii) localization of class II receptors in specialized structures on the cell surface may facilitate internalization.

Selective internalization. Studies on the distribution of receptors for insulin (*49*), epidermal growth factor (*50*), and IgE (*51*) demonstrate that these receptors are randomly distributed. Receptors occupied by ligand exhibit lateral mobility in the plane of the membrane. In a number of instances, signal transmission requires the lateral mobility of receptors. For example, mast cell histamine release results from IgE receptor cross-linking (*51*), and glucagon stimulation of adenylate cyclase appears to result from the association of mobile protein subunits in the membrane (*52*). Occupied receptors may also aggregate and form a patch that is internalized. The patch may become associated with a region of membrane referred to as a coated pit (*49*). The extent to which receptor-mediated internalization proceeds via coated pits is not known. Either all (*49*) or some (*50*) of receptor-bound epidermal growth factor is internalized by coated pits. α_2-Macroglobulin bound to the surface of fibroblasts appears to be internalized by coated pits (*49*). However, the physiological role of fibroblast uptake of α_2-macroglobulin is unclear. The fact that receptor-bound ligand is internalized suggests that ligand binding induces an alteration in receptor structure which results in internalization of receptor-ligand complexes. That this internalization is specific and restricted to only a subset of membrane components is inferred from the observation that on the same cell insulin will promote the loss of insulin receptors but not those of growth hormone (*53*).

These observations are fundamentally analogous to those of Berlin and colleagues (*54*) demonstrating that phagocytic activity results in the internalization of selected classes of membrane components. It has been suggested that endocytic stimuli, such as phagocytic particle or perhaps a ligand-receptor complex, may select a domain of lipids that leads to an altered membrane fluidity in the environment of the stimuli (*55*). This altered environment may allow for selective inclusion or exclusion of other classes of membrane proteins. Endocytosis of this area of membrane then results in the selective internalization of membrane components. Ukena and Berlin (*56*) have implicated microtubules as playing a controlling role in determining the lateral mobility of membrane proteins and thus by inference the selectivity of internalization, although the exact role of microtubules remains to be defined.

Specialized structures for internalization. Studies by Anderson *et al.* (*57*) show that the native distribution of LDL receptors is nonrandom and that the receptors are localized in coated pits. I have obtained evidence that at least 50 percent of α-macroglobulin–protease complex receptors on macrophage surfaces are localized in coated pits (*58*). That the native distribution of these receptors is nonrandom indicates that they are sequestered from the bulk of membrane proteins. That localization in coated pits is relevant to the function of class II receptors was demonstrated by the studies of Anderson *et al.* (*59*). These workers showed that fibroblasts from a hypercholesterolemic individual (J.D.) had receptors capable of binding but not of internalizing LDL. Studies of the topographical distribution of LDL receptors on these fibroblasts demonstrates that they are in fact excluded from coated pits. This observation is the most striking demonstration that receptor clustering is necessary for function. However, the mechanisms leading to receptor clustering, or the exact function of receptor localization in coated pits, is not understood. It is possible that aside from providing a specialized transport structure, an additional function of clustered receptors is to increase the valency of binding. Among the virtues of a multivalent binding system would be a lowered rate of dissociation (K_{-1}) of ligand-receptor complexes.

From the few studies on the distribution of other class II receptors that have been conducted it is difficult to determine whether the distribution observed is native or ligand-induced. For example, Roth *et al.* (*9*) observed that ferritin-IgG conjugates could bind in vitro to isolated plasma membrane of chicken yolk sac. In particular, when binding to the membrane occurred, the conjugate was localized in coated pits and only 20 percent of the coated pits contained ligand. However, since prior to membrane isolation the yolk sac plasma membrane had been exposed to IgG, it is unclear whether the localization of receptors in coated pits reflected the native distribution of receptors or was ligand-induced. Thus, although present studies indicate that class II receptors are localized in coated pits, further data are required to determine whether this is the native distribution of the receptors.

Conclusions and Prospectives

Receptors of disparate chemical nature can be grouped on the basis of function. In this article I have defined the characteristics of two categories of receptors—those whose major function is information transfer and those receptors whose function is primarily ligand uptake. Receptor classes are distinguishable on the basis of divalent cation dependency of ligand binding, topological distribution, and regulation of receptor

number. Class I receptors appear to be catabolized after ligand binding, whereas class II receptors are spared from catabolism.

The receptors within each category have similar characteristics that suggest possible mechanisms underlying receptor function. For example, most class II receptor-ligand interactions require divalent cations. This requirement may have arisen out of a necessity to modulate ligand-receptor interactions. If receptors have a fate independent of the bound ligand, then some mechanism must be able to modulate ligand-receptor affinity. One way to achieve such modulation would be to control the concentration of divalent cations. The concentration of divalent cations found in plasma would favor formation of ligand–class II receptor complexes. A low concentration of divalent cations in lysosomes (or incoming endocytic vesicles) would result in dissociation of ligand–class II receptor complexes, thus allowing for catabolism of ligand-independent receptors.

The classification of receptors by function points out areas for further study. For example, analysis of transferrin-mediated iron uptake by reticulocytes suggests that the transferrin receptor may be reutilized (*60*). However, binding of transferrin to reticulocyte receptors is independent of divalent cations, and the native distribution of transferrin receptors is random (*61*). Thus, it is unclear whether the transferrin receptor could best be described as a class I or class II receptor. If the transferrin receptor has behavior patterns similar to class II receptors, then one would predict that receptor function, in terms of either number or activity, would be unaltered by uptake of transferrin. This point is of interest because of the possibility that unregulated transferrin receptor function may be implicated in the etiology of diseases of iron overload, such as idiopathic hemochromatosis.

As more information on receptor characteristics becomes available, the number of categories may have to be expanded, modified, or perhaps even abandoned. However, the present classification of membrane receptors reveals insights into receptor physiology and may reflect a common evolutionary strategy.

Note added in proof: Three studies have recently appeared that directly demonstrate the recycling of membrane components (*66*).

References and Notes

1. S. C. Silverstein, R. M. Steinman, Z. A. Cohn, *Annu. Rev. Biochem.* **46**, 669 (1977); H. J.-P. Ryser, *Science* **159**, 390 (1968).
2. H. Metzger, in *Receptors and Recognition*, P. Cuatrecasas and M. F. Greaves, Eds. (Chapman & Hall, London, 1977), vol. 4, series A, p. 73.
3. C. R. Kahn, K. Baird, J. S. Flier, D. B. Jarrot, *J. Clin. Invest.* **60**, 1094 (1977).
4. T. Ishizaka, T. H. Chang, M. Taggert, K. Ishizaka, *J. Immunol.* **119**, 1589 (1977).
5. J. L. Goldstein and M. S. Brown, *J. Biol. Chem.* **249**, 5153 (1974).
6. M. J. Mahoney, L. E. Rosenberg, S. H. Mudd, T. Uhlendorf, *Biochem. Biophys. Res. Commun.* **44**, 375 (1971).
7. K. Ohlsson, *Acta Physiol. Scand.* **81**, 269 (1971); M. T. DeBanne, R. Requoeczi, J. Dolovich, *Biochim. Biophys. Acta* **336**, 165 (1973); H. Rinderknecht and M. Geokas, *ibid.* **295**, 233 (1973).
8. In mammalian systems, lysosomal glycosidases constitute a major class of glucose or mannose terminal glycoproteins. Ferritin is also internalized by this receptor [P. Stahl, J. S. Rodman, M. J. Miller, P. H. Schlesinger, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1399 (1978); P. Stahl, P. H. Schlesinger, E. Sigardson, J. S. Rodman, Y. C. Lee, *Cell* **19**, 207 (1980)].
9. T. F. Roth, J. A. Cutting, S. B. Atlas, *J. Supramol. Struct.* **4**, 527 (1976).
10. J. Kaplan and M. N. Nielsen, *J. Biol. Chem.* **254**, 7323 (1979).
11. R. L. Hudgin, U. E. Priler, G. Ashwell, R. S. Stockert, A. G. Morell, *ibid.* **249**, 5536 (1974).
12. S. K. Basu, J. L. Goldstein, M. S. Brown, *ibid.* **253**, 3852 (1978).
13. T. Roth, personal communication.
14. J. L. Goldstein, S. K. Basu, H. Brunschede, M. S. Brown, *Cell* **7**, 85 (1976).
15. J. Kaplan and M. N. Nielsen, *J. Biol. Chem.* **254**, 7329 (1979).
16. J. Kaplan, *Cell* **19**, 205 (1980).
17. T. Tanabe, U. E. Priler, G. Ashwell, *J. Biol. Chem.* **254**, 1038 (1979).
18. M. S. Brown and J. L. Goldstein, *Cell* **6**, 307 (1975).
19. D. Doyle, F. Hou, R. Warren, *J. Biol. Chem.* **254**, 6853 (1979).
20. R. S. Stockert, U. Gartner, A. G. Morell, A. M. Wolkoff, *ibid.* **255**, 380 (1980).
21. G. Carpenter and S. Cohen, *J. Cell Biol.* **71**, 159 (1976).
22. M. Ascoli and O. Puett, *J. Biol. Chem.* **253**, 4892 (1978).
23. C. R. Kahn and K. Baird, *ibid.*, p. 4900; J. Gillemann and O. Sonne, *ibid.*, p. 7857; J. R. Gavin, J. Roth, D. M. Neville, P. Demetts, D. N. Buell, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 84 (1974).
24. S. L. Gammeltoft, L. Kristensen, L. Sestoft, *J. Biol. Chem.* **253**, 8406 (1978).
25. F. R. Mayfield, P. J. A. Davies, L. Klempner, M. C. Willingham, I. Pastan, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5731 (1979).
26. H. J. Showell, D. Williams, E. L. Becker, P. H. Naccache, R. I. Sha'afi, *J. Reticuloendothel. Soc.* **25**, 139 (1979).
27. J. Niedel, S. Wilkinson, P. Cuatrecasas, *J. Biol. Chem.* **254**, 1070 (1979).
28. C. Bersky, J. Rivera, S. Mills, T. J. Triche, *J. Immunol.* **122**, 1926 (1979).
29. P. Youngdahl-Turner, I. S. Mellman, R. H. Allen, L. E. Rosenberg, *Exp. Cell Res.* **118**, 127 (1979).
30. J. Kaplan, C. Trummel, B. Goldberg, P. Robertson, *J. Clin. Invest.*, in press.
31. J. L. Goldstein, S. E. Dana, J. R. Faust, A. L. Beaudet, M. S. Brown, *J. Biol. Chem.* **250**, 8487 (1975).
32. M. S. Brown, S. E. Dana, J. L. Goldstein, *ibid.*, p. 4025.
33. S. Gordon, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 2707 (1977).
34. L. A. Sherman and J. Lee, *J. Exp. Med.* **145**, 76 (1977).
35. L. Sherman, personal communication.
36. R. M. Mahley, T. L. Innerarity, K. H. Weisgraber, D. L. Fry, *Am. J. Pathol.* **87**, 205 (1977).
37. J. L. Goldstein, R. G. M. Anderson, L. M. Buta, S. K. Basu, M. S. Brown, *J. Clin. Invest.* **59**, 1196 (1977).
38. J. L. Goldstein, Y. K. Ho, S. K. Basu, M. S. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 333 (1979).
39. J. L. Goldstein and M. S. Brown, *Annu. Rev. Biochem.* **46**, 897 (1977).
40. K. Ohlsson and I. Ohlsson, *Scand. J. Clin. Lab. Invest.* **34**, 349 (1974).
41. The mechanisms of uptake (transport) of metabolites such as nucleosides or amino acids are completely different from the mechanism of uptake from class II receptors. However, in some respects, transport elements (for example, adenosine) exhibit behavior similar to class II receptors; that is, their function is to facilitate the uptake of metabolites present in low concentration, and their number or activity is unaffected by incubation in high concentrations of metabolites. In many circumstances this unregulated receptor activity may lead to pathological consequences. In particular, unrestricted adenosine uptake may lead to toxic sequelae. To protect themselves, cells do not regulate adenosine uptake but convert adenosine into other purines [K. Ishii and H. Green, *J. Cell Sci.* **13**, 425 (1973); R. D. Berlin and R. A. Hawkins, *Am. J. Physiol.* **215**, 432 (1974); H. Green and K. Ishii, *J. Cell Sci.* **11**, 173 (1972); R. D. Berlin and R. A. Hawkins, *Am. J. Physiol.* **215**, 942 (1968)].
42. R. G. Anderson, personal communication.
43. R. A. Steinman, S. E. Brodie, T. A. Cohn, *J. Cell Biol.* **68**, 685 (1976).
44. Z. Werb and Z. A. Cohn, *J. Biol. Chem.* **247**, 2439 (1972); L. Warren and M. C. Glick, *J. Cell Biol.* **37**, 729 (1968).
45. B. Bowers and T. E. Olszewski, *J. Cell Biol.* **53**, 681 (1972).
46. S. H. Wollman and J. E. Lowenstein, *Endocrinology* **93**, 248 (1973).
47. J. E. Heuser and T. S. Reese, *J. Cell Biol.* **57**, 315 (1973).
48. R. Duncan and M. K. Pratten, *J. Theor. Biol.* **66**, 727 (1977).
49. J. Schlessinger, Y. Shechter, M. C. Willingham, I. Pastan, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2659 (1978).
50. H. T. Haigler, J. A. McKanna, S. Cohen, *J. Cell Biol.* **81**, 382 (1979).
51. T. Ishizaka and K. Ishizaka, *Prog. Allergy* **19**, 60 (1975).
52. M. Schramm, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1174 (1979).
53. M. A. Lesniak and J. Roth, *J. Biol. Chem.* **251**, 3730 (1976).
54. M. F. Tsan and R. D. Berlin, *J. Exp. Med.* **134**, 1016 (1971); J. M. Oliver, T. E. Ukena, R. D. Berlin, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 394 (1974).
55. R. D. Berlin and J. P. Fera, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1072 (1977).
56. T. E. Ukena and R. D. Berlin, *J. Exp. Med.* **136**, 107 (1972).
57. R. G. W. Anderson, J. L. Goldstein, M. S. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2435 (1976).
58. J. Kaplan, unpublished data.
59. R. G. W. Anderson, J. L. Goldstein, M. S. Brown, *Nature (London)* **270**, 695 (1975).
60. E. H. Morgan, in *Iron in Biochemistry and Medicine*, A. Jacobs and M. Worwood, Eds. (Academic Press, London, 1974), p. 29; R. G. Sephton and N. Kraft, *Cancer Res.* **38**, 1213 (1978); J. U. Larrick and P. Cresswell, *Biochim. Biophys. Acta* **583**, 483 (1979).
61. A. L. Sullivan, J. A. Grasso, L. R. Weintraub, *Blood* **47**, 133 (1976); R. T. Parmley, F. Ostroy, R. A. Gams, L. DeLucas, *J. Histochem. Cytochem.* **27**, 681 (1979).
62. V. Soman, *Nature (London)* **272**, 829 (1978).
63. B. Rubalcava and M. Rodbell, *J. Biol. Chem.* **248**, 3821 (1973).
64. P. M. Hinkel and A. L. Tashjian, *Biochemistry (USSR)* **14**, 3845 (1976).
65. This is surmised from the observation that fMet-Leu-Phe of C_5A induced chemotaxis is independent of external Ca^{2+} or Mg^{2+} [W. A. Marasco, E. L. Becker, J. M. Oliver, *Am. J. Pathol.*, in press].
66. Y. J. Schneider, P. Tulkens, C. De Duve, A. Trouet, *J. Cell Biol.* **82**, 466 (1979); L. Thilo and G. Vogel, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1015 (1980); W. A. Muller, R. A. Steinman, Z. A. Cohn, *J. Cell Biol.* **86**, 304 (1980).

67. I thank J. Jannace and C. Clayton for typing the manuscript, and M. J. Osborn, R. D. Berlin, P. A. Ward, and M. L. Tanzer for advice and suggestions. This work was supported by NIH grants HL 21120 and HL 23376. The author is a recipient of a Research Career Development Award (HL 00598) from NIH.