Birgit H. Satir, Series Editor

MODERN CELL BIOLOGY

Volume 1

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Foreword

The goal of this series is to provide researchers in cell and molecular biology with comprehensive articles that review areas of current significance and raise pertinent questions about future directions. We seek to publish articles that look to the future but provide lasting value. This is a need that is not being fully satisfied by any of the existing publications.

These are exciting times for the cell biologist. Our own advances and insights are being stimulated and enriched not only by research done by fellow cell biologists, but by research from our colleagues in such diverse but increasingly interconnected fields as molecular biology, developmental biology, biophysics, immunology, genetics, bioengineering, etc. This gives a particular urgency to the task of creating a review series truly reflective of the continuing evolution of our understanding of the biology of the cell. To help us do this we have gathered together an advisory board that is broadly based in both techniques and interests, as well as international in its composition, to reflect a diversity of breadth and depth that this series seeks to provide.

The papers in this series will be critical reviews of specific areas in cell biology and related fields, including personal perspectives, and general reviews related to the boundaries of the field or the application of new methods to cell biological problems. From time to time, a single volume will be organized as an in depth exploration of an area of importance in cell biology. An example of this is Volume 2, Spatial Organization of Eukaryotic Cells, a special volume in honor of one of the distinguished leaders in the field, Keith R. Porter.

We hope readers find these reviews both useful and exciting.

Birgit Satir
December 1982

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Receptor-Mediated Endocytosis

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I. INTRODUCTION

It has been nearly 90 years since Overton [1] first carried out his classical studies on the permeability properties of cellular membranes. Many inves-

tigators have since confirmed that the lipid composition of biological membranes renders them impermeable to either highly charged molecules or to molecules larger than 400 daltons [2]. Only if the membrane is altered through the insertion of specific types of membrane molecules, which are usually proteins, is the cell able to selectively take up extracellular molecules that ordinarily are unable to penetrate the lipid bilayer. It is this event that transforms the membrane into a selective barrier. In addition, cells have developed several different mechanisms for internalizing portions of the extracellular environment and to deliver the trapped contents to various compartments within the cell. This process, called "endocytosis," is adapted either to ingest small portions of the extracellular environment (pinocytosis) or to ingest large, particulate material (phagocytosis). Although the two processes achieve the same end, the internalization of material from the extracellular space, the physiologic mechanisms involved are quite distinct [3].

In this review, we wish to restrict our discussion to the endocytosis of molecular material (pinocytosis), a process that requires the invagination of small regions of the surface membrane to form a vesicular compartment that contains a portion of extracellular fluid. With few exceptions (see Endocytosis), this type of endocytosis is a constitutive process that takes place at a constant rate for a given cell under defined environmental conditions. Steinman et al [4–6] used horseradish peroxidase as a molecular marker to measure the rate of endocytosis in macrophages and L cells in culture. They found that for both cell types, 10^6 cells internalize 10^{-4} ml of culture media each hour. In the same time interval, macrophages internalize 186% and L cells 54% of their cell surface [6]. They also found that among different fibroblasts, the rate of endocytosis is approximately the same [5], although the growth conditions of the cells can influence this rate.

The endocytic process displayed by these cells lacks specificity and is inefficient. Molecules are internalized in proportion to their concentration in the extracellular environment without regard to their metabolic importance to the cell. To overcome this constraint, cells have devised ways to increase the effectiveness of endocytosis. They synthesize receptors that function in the plasma membrane to recognize, concentrate, and internalize by endocytosis specific groups of extracellular macromolecules. The coupling of membrane receptor activity to macromolecule internalization is called receptor-mediated endocytosis (RME). Over 20 different macromolecules or macromolecular complexes have now been shown to be internalized by RME [7, 8]. The diversity of this class of molecules, from transport proteins to hormones, suggests that the cell utilizes RME to meet a variety of different physiological needs.

Receptor-mediated endocytosis is a multiphase process. Specific cell surface receptors must be located on the cell surface. In addition, the cell must

be able to carry out endocytosis, a process that often involves differentiated regions of the cell surface. The receptors must be located over those regions of membrane that are undergoing endocytosis to achieve efficient uptake of the bound molecule. Once inside the cell, the contents of the endocytic vesicle and the receptors must be segregated so that receptors and their specific ligand can be delivered to separate intracellular targets. Often two or more molecules can enter the cell through the same endocytic vesicle. They too must be segregated so that they can eventually go to different intracellular compartments.

This highly organized cellular process has been studied by many investigators using several different cell systems. Necessarily, this review can neither cover every aspect of endocytosis nor comprehensively review receptor biology [see 3, 7, 9–12 for reviews]. Even an exhaustive compilation of the literature on RME will not be achieved. Our purpose in this review is to bring together diverse experimental findings and use this information to construct various models that will be useful for identifying the salient problems in this field, and we hope, to indicate new directions for future research.

II. RECEPTORS

A. Definition

Cell surface receptors are able to recognize and bind with high affinity specific subsets of extracellular macromolecules; furthermore, the binding step usually elicits a cellular response. In the case of those receptors involved in RME, a major response is the internalization of the ligand. This may be preceded by the generation of a signal that alters cellular metabolism (eg, polypeptide hormone receptors), or the internalized ligand may be utilized by the cell for specific metabolic needs. In either case, ligand binding is a physiologically important event.

Ligand-receptor interaction is specific and involves only one family of homologous extracellular molecules and one set of plasma membrane proteins. These receptors usually have been found to be a single protein or protein-protein complex [eg, see 13–17]. Moreover, the binding of the specific ligand depends on characteristic ionic and pH conditions. Ligand-receptor interactions have often been further defined by assessing how the specific modification of either the receptor or the ligand inactivates the binding step.

These receptors can therefore be defined by their molecular properties, the conditions for ligand binding, and their ability to mediate a specific physiologic event. It is this last property that has usually been responsible for their initial detection. For example, LDL receptors were discovered be-

cause of their ability to regulate intracellular cholesterol metabolism [28, 29]. Similarily, the asialoglycoprotein receptor [30] and the lysosomal enzyme receptor [31], to mention two, were first detected as a result of their physiological activity, not their binding properties.

Even though the physiological response is the single most important criterion for establishing the identity of a specific ligand-receptor interaction, often receptor activity must be studied under conditions where the physiologic response cannot be measured. This is particularly true when trying to detect receptors in fractionated cells or in cells that have been treated with fixatives like formaldehyde or gluteraldehyde. In these situations, the identification of receptor activity has to be based on the properties of ligand binding. These properties must be the same as those established for the intact, responsive cell. Thus, it is not sufficient to measure just ligand-specific displaceable binding (the competition between radiolabeled ligand and excess unlabeled ligand for the receptor). Criteria such as time dependence, ionic and chemical requirements, and cell specificity must also be established [32–34].

Analysis of ligand-receptor interaction is sometimes made difficult by the fact that under physiological conditions binding is not characteristically a bimolecular interaction:

$$(R + L \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} RL)$$

This is because in the intact cell often the rate of dissociation (K_{-1}) cannot be measured. There may be several different reasons for why a ligand does not dissociate after binding: 1) Ligand binding may be a multivalent step whereby a single ligand can bind to more than one receptor. Galactose terminal glycoprotein [13] and membrane-specific antibodies are examples of these types of ligands. Since the binding of a single ligand to multiple receptors exhibits a rate of dissociation that is a power of the valence of the ligand (that is, $(K_{-1})^n$, where n is the valency and K_{-1} the rate of dissociation), for multivalent ligands the rate of dissociation is so slow that binding is essentially irreversible. 2) Upon binding, ligand may become covalently attached to the receptor, thus precluding a dissociation step. Evidence indicates that some of the cell-bound insulin [18], epidermal growth factor [19–21], and thrombin [20, 22] becomes covalently attached to their receptors. In some cases, covalent binding of ligand to receptor may not be physiological but may represent an artifact of the radioactive labeling technique used to tag the ligand [23]. 3) Once the ligand binds to the receptor, the ligand-receptor complex is internalized. Ligands such as LDL are internalized so rapidly that there is not time for dissociation to take place. This behavior is common for the majority of those receptors listed in Table II,

for epidermal growth factor [24], and for human choriogonadotropin [25]. Therefore, in the case of receptors involved in RME, often conventional methods for determining affinity are not applicable. Moreover, it may be impossible to establish whether ligand-receptor interactions exhibit cooperativity or are multivalent. Recently, Wiley and Cunningham [26] devised a particularly useful method for receptor analysis that depends on establishing binding conditions under steady-state conditions rather than at equilibrium. Such a methodology can be used to derive expressions for measuring both the rate of endocytosis and the rate of ligand-induced receptor loss.

B. Physiology

In 1981 Kaplan [27] reviewed the physiologic properties of those cell surface receptors that are involved in specific macromolecule binding. He noted that these receptors could be assigned to one of two catagories: 1) receptors that transport molecules into the cell for further metabolic processing (class II), or 2) receptors that function to directly alter cell behavior or metabolism after ligand binding (class I). Class I receptors can be further divided into those that are able to internalize their ligand (class IA) and those that do not internalize ligand (class IB). Tables I and II list the members of each class along with some of their biochemical properties. The members of each class show similarity in receptor reutilization, surface distribution, ion requirements for ligand binding, and receptor regulation.

C. Reutilization

One of the hallmarks of the class II receptors is that the receptor is reutilized multiple times during its lifetime. Since these receptors function to internalize transport proteins or to clear undesirable macromolecules from the extracellular space, it stands to reason that receptor reutilization would be necessary to maintain a steady-state internalization of the molecules. In many cases, the ligand internalized by these receptors is catabolized in lysosomal compartments; alternatively, the ligand is stored or transported through the cell to the extracellular space. Invariably, the receptor escapes degradation, returns to the cell surface, and mediates the internalization of more ligand (see Receptor Recycling).

In contrast, most class I receptors are not reutilized. Upon binding, the ligand is internalized and the ligand-receptor complex goes to the lysosomal compartment. This process results in an effective reduction of receptors from the cell surface. "Down-regulation" of surface receptors causes the cell to lose its sensitivity to class I molecules. To illustrate, gonadotropin induces steroid synthesis in target cells. However, the gonadotropin effect requires that the ligand and the receptor exist as a complex on the cell surface. Removal of surface receptor-bound ligand by chemical treatment or by internalization

TABLE I. Class I Receptors

Ligand	Function	Regulation of receptor	Surface distribution	Selected references
Class IA Receptors				
Chorio-gonadotropin	Hormone production	Down-regulation	Unknown	25, 35
Epidermal growth factor	Mitosis	Down-regulation	Random or clustered in coated pits	24, 26, 188, 252, 253
Glucagon	Metabolic activity	Down-regulation	Unknown	254
Insulin	Metabolic activity	Down-regulation	Random	255–259
Thyroid-releasing hormone	Hormone secretion or synthesis	Down-regulation	Unknown	260
Somatostatin	Mitosis	Down-regulation	Unknown	261
Growth hormone	Mitosis	Down-regulation	Unknown	36
f-met-leu-phe, polymorpho-nuclear leukocyte	Ion transport, chemotaxis	Down-regulation	Random	39, 262, 263
C_{5a}, polymorpho-nuclear leuko-cyte	Ion transport, chemotaxis	Down-regulation	Unknown	262
Class IB receptors				
Acetylcholine	Ion transport, muscle contraction	Increase in dissociation rate	Clustered	61
Catecholamine	cAMP	Decreased affinity	Unknown	62, 270
IgE, mast cells	Histamine secretion	None	Random	271, 272

results in a reduction in the synthesis of steroid hormone [35]. Down-regulation also affects the sensitivity of cells to a subsequent exposure to the ligand. Studies on the internalization of epidermal growth factor [24, 36], human growth hormones [37], and choriogonadotropin [25] indicate that internalization of ligand is correlated with receptor loss. It is less clear whether insulin can down-regulate its receptor [38]. Perhaps the effect of insulin on receptor reutilization depends on the cell type.

A noted exception among the class I receptors is those for the polypeptide F-met-leu-phe in polymorphonuclear leukocytes. This receptor, which causes

TABLE II. Class II Receptors

Ligand	Function	Regulation of receptor	Surface distribution	Divalent cations required for binding	Low pH sensitivity of binding	Reference
Low-density lipoprotein	Supplies cholesterol	Cholesterol	Coated pits	+	+	29, 58, 125
α_2-Macroglobulin-protease complex	Removes injurious agents	None	Coated pits	+	+	44, 205, 206
N-acetylglucosamine/mannose terminal glycoproteins	Removes injurious agents	None	Coated pits	+	+	44, 264
Galactose terminal glycoproteins	Removes injurious agents	None	Coated pits	+	+	13, 128,232
IgG, oocyte	Fetal immunity	None	Coated pits	+	+	45
Vitellogenin	Source of protein for embryo	None	Unknown	+	+	273
Fibrin	Removes injurious agents	None	Unknown	+	Unknown	265
Cobalamin, cobalamin II	Supplies vitamin B12	None	Microvilli	+	Unknown	266, 267
Mannose-6-phosphate terminal glycoproteins	Delivers lysosomal enzymes to lysosome	None	Coated pits	−	+	9, 46, 50
IgG, small intestine	Newborn immunity	None	Coated pits	−	−	51, 53, 54
Transferrin	Iron uptake	Iron	Random	−	−	56, 268, 269
Semliki forest virus	Virus infection	None	Random	−	−	273

cells to orient in a gradient of the polypeptide, appears to be capable of recycling. In the presence of the polypeptide, the majority of these receptors leave the cell surface within 20 minutes [39]. The receptors remaining on the cell surface are able to continually internalize the polypeptide by a receptor-mediated process. If the polypeptide is then removed from the culture media, the receptors return to the cell surface without a requirement for protein synthesis [40]. At the plateau level there may be a steady-state internalization and return of receptor to the cell surface in the presence of ligand, but after ligand removal all of the internalized receptors return to the cell surface. These cells apparently contain a mechanism for preventing the receptor from getting degraded.

D. Surface Distribution

Where studied, nearly all receptor-mediated processes occur through coated pits (see Endocytosis). Some of these receptors are initially randomly distributed in the membrane and require ligand binding before moving into coated pits. Other receptors appear to be preclustered in coated pits. All of those examples of apparent preclustered receptors belong to the class II family of receptors. Evidence exists that the majority of the receptors for LDL in human fibroblasts [41, 42], asialoglycoprotein in hepatocytes [43], mannose terminal glycoprotein and α_2-macroglobulin protease complex in macrophages [44], IgG in chicken oocytes [45], mannose phosphate terminal glycoprotein in fibroblasts [46], and ferritin in reticulocytes [47] are prelocalized in coated pits. It has not been determined in each case that ligand binding did not induce movement into the coated pit during the period of labeling [see 48, 49]; however, in the case of LDL and IgG this is unlikely. The possible mechanisms of receptor clustering in coated pits are considered later.

E. Ion Requirements for Binding

In contrast to class I receptors, ligand binding to class II receptors is usually sensitive to cation concentrations and pH. Whereas most of the receptors that require calcium for binding also dissociate at low pH, some receptors—for example, those for mannose phosphate terminal glycoproteins [50]—are strictly pH-sensitive. Since class II receptors undergo reutilization during internalization, the sensitivity of the receptor to low pH and/or to calcium may play a role in this process (see Receptor Recycling).

Although most class II receptors exhibit a lower ligand affinity at low pH, the opposite is true for the IgG receptors in neonatal intestinal epithelial cells. The lower the pH, the more tightly this ligand binds to its receptor [51–53]. This seems to be a specialization that permits the receptor to function in the relatively acidic environment of the neonatal intestine. Interestingly, once internalized, the IgG appears to remain bound to its membrane and is trans-

ported to the lateral border of the cell. At this site, exocytosis takes place and the IgG dissociates at the higher pH (7.2 to 7.4) of this compartment [54, 55].

A class II receptor that is a total exception to this rule is the receptor for transferrin. Neither calcium nor pH affects the binding of transferrin to its receptor. Once internalized, transferrin is able to release its iron, possibly because of the low pH environment of the endocytic compartment or the lysosome [56]; however, alternative interpretations have been proposed [57]. Even though iron is removed from the transferrin in the cell, the apotransferrin does not accumulate and is not degraded; rather, it is released intact from the cell [56]. Thus, this receptor may function to transport transferrin to a compartment where the iron is deposited and then return during membrane recycling to the cell surface.

The ionic requirements for class II receptor activity seem to be an important aspect of their ability to function as transport receptors: 1) Receptors that accumulate extracellular ligand without any depletion of receptors from the cell surface depend on either divalent cation or neutral pH for ligand binding. 2) Among the receptors involved in transcellular transport, whether it be to another surface of the cell or to the same surface from which it originated, ligand binding is neither low pH-sensitive nor low cation-sensitive. 3) Receptors that function primarily to direct ligands to various intracellular compartments, such as the mannose-6-phosphate receptor, are sensitive to low pH but not to low divalent cation concentration. More work is required to understand how the sensitivity to various ions in the environment regulates receptor activity.

F. Regulation

The mode of regulation of class I receptors is different from that of class II receptors. At least some of the class I receptors (class IA) appear to be regulated by ligand-induced loss of receptors during internalization. Such receptors exhibit a high affinity for their respective ligands; however, it is unclear whether or not the ligand-induced down-regulation is a specific cellular response that is designed to decrease the sensitivity of the cells to the ligand. Class II receptors, in contrast, appear to be regulated by the metabolic products derived from the internalized ligand. (In most cases these receptors are not regulated at all. See Table II.) As an example, the LDL receptor is regulated by the cholesterol liberated from the degradation of LDL in the lysosome [58]. Likewise, iron may be able to regulate the number of transferrin receptors on the cell surface [59]. Iron seems to decrease the rate of transferrin receptor synthesis without altering the rate of receptor degradation.

Some class I receptors (class IB) do not appear to be regulated by either the binding of ligand or the breakdown products of that ligand. A prime