ANNUAL REVIEW OF CELL BIOLOGY

VOLUME 1, 1985

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ANNUAL REVIEWS INC. 4139 EL CAMINO WAY PALO ALTO, CALIFORNIA 94306 USA

ANNUAL REVIEWS INC.
Palo Alto, California, USA

International Standard Serial Number: 0743-4634
International Standard Book Number: 0-8243-3101-X

TYPESETTING BY AUP TYPESETTERS (GLASGOW) LTD., SCOTLAND
PRINTED AND BOUND IN THE UNITED STATES OF AMERICA

ANNUAL REVIEW OF CELL BIOLOGY

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PREFACE

The first volume of this new series of Annual Reviews came into being as a result of the convergence of two major currents of interest connected with the relatively young but rapidly advancing field of Cell Biology. One of these currents had its source in Annual Reviews Inc. (ARI), whose mission is to review the literature of significant areas of contemporary research. Individual proposals for an annual review of Cell Biology had been received from a number of scientists as early as 1969, and these led to the decision in 1983 by the Board of Directors of Annual Reviews Inc. that the time had come for a new review in this field. The other current came from the American Society for Cell Biology whose 1982 president, Marilyn Farquhar, and publication committee, led by Richard McIntosh, felt that the degree of development of the field required an annual review in addition to regular journals. Fortunately the two currents converged and, after preliminary discussions, an organizing meeting was held in October 1983 at the Annual Reviews headquarters in Palo Alto, California. Those in attendance included Winslow Briggs (Stanford University), William Brinkley (Baylor University), Marilyn Farquhar (Yale University), Peter Hepler (University of Massachusetts), Richard Hynes (MIT), Marc Kirschner (UCSF), David Luck (Rockefeller University), George Palade (Yale University), David Prescott (University of Colorado), Jean Paul Revel (California Institute of Technology), James Spudich (Stanford University), and several ARI staff members.

This meeting, which was chaired by Winslow Briggs, representing the Board of Directors of Annual Reviews, worked out the list of authors and chapters for the present volume. On that occasion, I was asked to become Editor of the Annual Review of Cell Biology. I accepted and was fortunate in obtaining the assistance of a competent and helpful Editorial Committee as well as the support of the editorial staff of Annual Reviews. From the beginning, I decided that close contact with the American Society for Cell Biology should be maintained, so as to benefit from the Society's suggestions as to appropriate topics and authors for future volumes.

The currents mentioned above were effective in giving birth to this new Annual Reviews series, but the real forces behind the move were the spectacular growth of Cell Biology over the last two or three decades, and the broad and close contacts it has established with the equally young fields

of Molecular Biology and Molecular Genetics, as well as with such old and traditionally established disciplines as Biochemistry, Genetics, and Physiology.

The broadest and most intimate of these contacts are leading, in fact, to a gradual merger of the fields of Cellular and Molecular Biology, Molecular Genetics included. We are witnessing a powerful syncretic movement that has already generated a common conceptual and methodological ground for many basic biological sciences, which until recently were separated into distinct disciplines on the basis of approaches used or domains covered. We can consider this syncretic process as the logical expression of our recently acquired knowledge that the unity of organization of living systems extends far beyond the small and large molecules of Biochemistry to reach the level of the macromolecular assemblies, cell organs, and cells of Cellular and Molecular Biology. The fact that so many pieces of complex biochemical equipment that perform highly integrated metabolic reactions have been extensively conserved through evolution makes even more impressive than originally thought the unity of the foundation on which all living systems are built.

About three decades ago, the contact established and maintained with Biochemistry was particularly important for the subsequent development of Cell Biology. Over the last decade the nascent merger with Molecular Biology has given every sign of being even more important and fruitful than the first close contact. The new development is rooted in more than common technology; it stems from the realization that the genome can function only within the structural and chemical framework of the cell, a framework produced and modulated by the genes of distant ancestors, preserved by temporal continuity through cell generations, and inherited together with a daughter genome by each daughter cell at each cell division. The framework cannot be maintained without the genome, and the genome cannot function or even survive without the cellular framework. In the future, cellular and molecular biology, molecular genetics included, promise to be as inextricably dependent on one another as the genome is on the cellular framework that acts as the equipment for its expression, as its protective prison, and as its access to potential immortality. The *Annual Review of Cell Biology* will make a sustained effort to cover the merging fields of Cellular and Molecular Biology to the widest extent practically possible.

The importance assumed by Cell Biology in all fundamental biological sciences is clearly attested by the fact that topics developed (or under development) in Cell Biology have been reviewed in recent years in the Annual Reviews of Biochemistry, Genetics, Physiology, Plant Physiology, and Biophysics and Bioengineering. The publication of a separate *Annual*

Review of Cell Biology is justified on two accounts. First, the research community in Cellular and Molecular Biology is large enough and diverse enough in its specific interests to need an annual review that covers the whole field. Second, there is enough interest in Cellular and Molecular Biology in adjacent areas to warrant convenient access to a review of the entire field as it develops year by year. These grounds do not restrict in any way the publication of reviews on Cell Biology topics in other Annual Reviews series. Since the object is efficient communication among research scientists in the general area of basic biological sciences, all outlets should be useful. The *Annual Review of Cell Biology*, however, may prove to be the most useful means of communication within the field and immediately outside it. This is the goal of the new series.

GEORGE E. PALADE
EDITOR

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RECEPTOR-MEDIATED ENDOCYTOSIS: Concepts Emerging from the LDL Receptor System

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CONTENTS

INTRODUCTION

The concept of *receptor-mediated endocytosis* was formulated in 1974 to explain the observation that regulation of cellular cholesterol metabolism

0743–4634/85/1115–0001$02.00

depended on the sequential cell surface binding, internalization, and intracellular degradation of plasma low density lipoprotein (LDL) (Goldstein & Brown 1974, Goldstein et al 1976). This uptake mechanism was postulated on the basis of biochemical studies; it was soon verified morphologically when the receptors for LDL were observed to be clustered in coated pits that pinched off from the surface to form coated vesicles that carried the LDL into the cell (Anderson et al 1976, 1977a).

Coated pits and coated vesicles had been recognized by electron microscopy in the mid-1960s (Roth & Porter 1964, Fawcett 1965, Friend & Farquhar 1967). Their role in receptor-mediated endocytosis was appreciated a decade later as a result of the convergence of twin events: 1) the demonstration that coated vesicles were the sites at which LDL receptors were concentrated, and 2) the demonstration by Pearse (1975) that a single protein, clathrin, formed the cytoplasmic coat, an observation that provided a biochemical definition of coated vesicles. The biological implications of receptor-mediated endocytosis were vividly underscored by the finding that genetic defects in the LDL receptor preclude cellular uptake of LDL, producing hypercholesterolemia and heart attacks (Brown & Goldstein 1984).

During the last decade, receptor-mediated endocytosis was recognized as a mechanism by which animal cells internalize many macromolecules in addition to LDL (Goldstein et al 1979a, Pastan & Willingham 1981, Bretscher & Pearse 1984). The process is initiated when receptors on the cell surface bind macromolecules and slide laterally into clathrin-coated pits. Within minutes the coated pits invaginate into the cell and pinch off to form coated endocytic vesicles. After shedding their clathrin coats the vesicles fuse with one another to form endosomes whose contents are acidified by ATP-driven proton pumps (Tycko & Maxfield 1982, Helenius et al 1983, Pastan & Willingham 1983). Within the endosome the ligand and receptor part company. Often, but not always, the ligand is carried to lysosomes for degradation, while the receptor cycles back to the cell surface to bind new ligand (Brown et al 1983).

More than 25 specific receptors have been observed to participate in receptor-mediated endocytosis. These include receptors for transport proteins that deliver nutrients to cells, such as the cholesterol-carrying lipoprotein LDL, the iron transport protein transferrin, and the vitamin B_{12} transport protein transcobalamin II. Receptor-mediated endocytosis also applies to many nontransport plasma proteins, including asialoglycoproteins, α-2-macroglobulin, and immune complexes. Moreover, the process mediates the cellular uptake of lysosomal enzymes, which occurs when these enzymes bind to receptors that recognize mannose-6-phosphate residues uniquely attached to this class of proteins. Certain viruses and

toxins use receptor-mediated endocytosis to enter cells, apparently by binding opportunistically to receptors that normally function in the uptake of other substances.

Protein growth factors, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), as well as classic polypeptide hormones, such as insulin and luteinizing hormone, also enter cells by receptor-mediated endocytosis. The same receptors that mediate endocytosis of these proteins mediate their physiologic actions. However, frequently cellular entry of the ligand does not seem to be required for the action of the growth factor or the hormone. Rather, the entry mechanism functions in the rapid control of receptor number and in the removal of the growth factor or hormone from the circulation (Carpenter & Cohen 1979, Terris et al 1979).

Progress in this field has been rapid. Within a single year—1984—complementary DNAs (cDNAs) for five different coated pit receptors were isolated, and their nucleotide and corresponding amino acid sequences were determined (Mostov et al 1984, Ullrich et al 1984, Russell et al 1984, Yamamoto et al 1984, McClelland et al 1984, Schneider et al 1984, Holland et al 1984). The cDNA cloning and structure of a sixth coated pit receptor, the insulin receptor, was reported early in 1985 (Ullrich et al 1985, Ebina et al 1985). In this review we summarize the information that is emerging from study of the amino acid sequences of the receptor proteins, with emphasis on the LDL receptor.

PATHWAYS OF RECEPTOR-MEDIATED ENDOCYTOSIS

Entry Into Coated Pits

The various pathways of receptor-mediated endocytosis share one common feature: in each case the receptors move to coated pits and coated vesicles. However, there are differences in the mechanisms that trigger movement to coated pits as well as differences in the routes the ligands and receptors follow after entering the cell. We can divide the process of receptor-mediated endocytosis into subcategories according to these differences, as described below.

The first distinction is whether the receptors spontaneously move to coated pits and enter cells continuously (even in the absence of ligand), or whether the receptors wait on the surface until a ligand is bound, whereupon they are captured by coated pits. The receptors in the first category include those for LDL (Anderson et al 1982, Basu et al 1981), transferrin (Hopkins & Trowbridge 1983, Hopkins 1985), α-2-macroglobulin (Hopkins 1982, Via et al 1982), asialoglycoproteins (Wall et

al 1980, Berg et al 1983), and insulin (Krupp & Lane 1982). Conversely, the receptor for EGF is diffusely distributed on the cell surface, and is not trapped in coated pits unless it is occupied with ligand (Schlessinger 1980, Dunn & Hubbard 1984).

The propulsive force for movement of receptors to coated pits may be simple diffusion, or it may involve a more directed type of propulsion (Bretscher 1984). The rate of diffusion of receptors on cell surfaces is sufficiently fast in itself to explain movement into coated pits (Goldstein et al 1981, Barak & Webb 1982). However, considerable evidence suggests that membrane lipids are continuously flowing toward coated pits (Bretscher 1984). This lipid flow may carry membrane proteins along passively (Bretscher & Pearse 1984, Hopkins 1985), but why are only certain cell surface proteins trapped in coated pits? One possibility is that receptors are marked for such entry by the attachment of prosthetic groups. Many receptors (such as those for transferrin, asialoglycoproteins, EGF, PDGF, and insulin) have phosphate groups attached to serine, threonine, or tyrosine residues in their cytoplasmic domains (see Table 1 in Brown et al 1983).

Recent attention has focused on phosphorylation or dephosphorylation as a potential mechanism for signaling entry, perhaps through induction of receptor binding to clathrin, the protein that covers the cytoplasmic surface of coated structures. Phosphorylation of the receptors for EGF (Hunter 1984), transferrin (Klausner et al 1984), and insulin (Jacobs et al 1983) can be enhanced by treatment of cells with phorbol esters, which activate protein kinase C. Phorbol esters cause transferrin receptors in K562 cells to become trapped within the cell, which suggests that phosphorylation either increases the rate of their cellular entry or slows their return to the cell surface, or both (Klausner et al 1984).

A few receptors undergo acylation of cysteine residues with fatty acids, but this modification does not apply to all receptors that participate in endocytosis. Moreover, in the one case that has been studied in detail, that of the transferrin receptor, the turnover of the fatty acid moiety is much slower than the internalization rate (Omary & Trowbridge 1981), which implies that acylation-deacylation does not occur during each recycling event.

Intracellular Routes

A second variation in the systems of receptor-mediated endocytosis is the fate of the ligand and receptor. It appears that all endocytotic receptors enter cells in the same coated pits, and are delivered to the same acidified endosomes (Pastan & Willingham 1981, Via et al 1982, Carpentier et al 1982). Thereafter, the pathways diverge. The receptor-ligand complex may

follow one of four routes, which are discussed below and illustrated schematically in Figure 1.

ROUTE 1: RECEPTOR RECYCLES, LIGAND DEGRADED This pathway is the classic one described for the endocytosis of LDL, asialoglycoproteins, and α-2-macroglobulin, as well as for insulin and luteinizing hormone. In following this route, ligands dissociate from their receptors within the endosome, apparently as a result of the drop in pH (Brown et al 1983, Helenius et al 1983). The ligand is carried further to lysosomes, where it is degraded. The receptor leaves the endosome, apparently via incorporation into the membrane of a vesicle that buds from the endosome surface. These recycling vesicles may originate as tubular extensions of the endosome, which gather receptors and then pinch off from the main body of the endosome (Geuze et al 1983, 1984). After their return to the surface, LDL receptors are said to remain clustered so that they can be incorporated rapidly into newly formed coated pits (Robenek & Hesz 1983). Conversely,

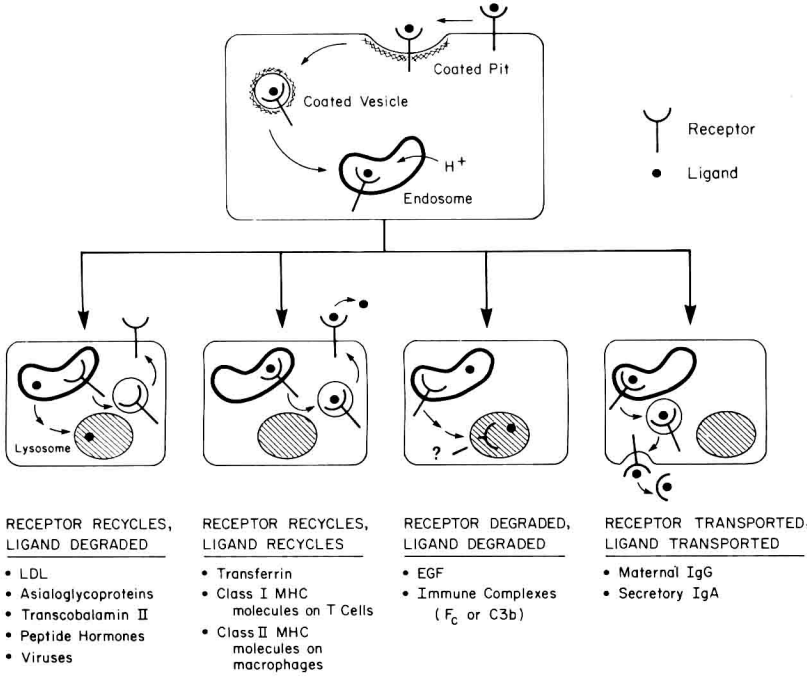

Figure 1 Four pathways of receptor-mediated endocytosis. The initial steps (clustering of receptors in coated pits, internalization of coated vesicles, and fusion of vesicles to form endosomes) are common to the four pathways. After entry into acidic endosomes, a receptor-ligand complex can follow any of the four pathways shown in the figure.