Membrane recycling

Ciba Foundation symposium 92

1982

Pitman

Membrane recycling

The Ciba Foundation is an international scientific and educational charity. It was established in 1947 by the Swiss chemical and pharmaceutical company of CIBA Limited—now CIBA-GEIGY Limited. The Foundation operates independently in London under English trust law.

The Ciba Foundation exists to promote international cooperation in medical and chemical research. It organizes international multidisciplinary meetings on topics that seem ready for discussion by a small group of research workers. The papers and discussions are published in the Ciba Foundation series.

The Foundation organizes many other meetings, maintains a library which is open to all graduates in science or medicine who are visiting or working in London, and provides an information service for scientists. The Ciba Foundation also functions as a centre where scientists from any part of the world may stay during working visits to London.

Membrane recycling

Ciba Foundation symposium 92

1982

Pitman

ISBN 0 272 79656 5

Published in October 1982 by Pitman Books Ltd, London.
Distributed in North America By CIBA Pharmaceutical Company (Medical Education Administration), Summit, NJ 07006, USA

Suggested series entry for library catalogues:
Ciba Foundation symposia

Ciba Foundation symposium 92
x + 310 pages, 58 figures, 12 tables

British Library cataloguing in publication data:
Membrane recycling.—(Ciba Foundation symposium; 92)
1. Cell membranes—Congresses
I. Evered, David II. Collins, Geralyn M.
III. Series
574.87'5 QH601

Text set in 10/12 pt Linotron 202 Times, printed and bound in Great Britain at The Pitman Press, Bath

Contents

Symposium on: Membrane recycling, held at the Ciba Foundation, London, 19–21 January 1982
Editors: David Evered (Organizer) and Geralyn M. Collins

Participants

P. F. BAKER F.R.S. Department of Physiology, King's College London, Strand, London WC2R 2LS, UK

A. D. BLEST Department of Neurobiology, Research School of Biological Sciences, Box 475 PO, Canberra City, ACT 2601, Australia

D. BRANTON Cell and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, USA

M. S. BRETSCHER MRC Laboratory of Molecular Biology, Medical Research Council Centre, University Medical School, Hills Road, Cambridge CB2 2QH, UK

Z. A. COHN Cellular Physiology and Immunology Laboratory, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

P. CUATRECASAS Department of Molecular Biology, Wellcome Research Laboratories, Burroughs Wellcome Co., 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709, USA

R. T. DEAN Department of Applied Biology, School of Biological Sciences, Brunel University, Uxbridge, Middlesex UB8 3PH, UK

M. G. FARQUHAR* Section of Cell Biology, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut 06510, USA

M. J. GEISOW Biophysics Division, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

J. L. GOLDSTEIN Department of Molecular Genetics, University of Texas Health Sciences Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235, USA

* Unable to attend the symposium

A. HELENIUS Section of Cell Biology, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut 06510, USA

C. R. HOPKINS Department of Histology and Cell Biology, The Medical School, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK

A. L. HUBBARD Department of Cell Biology and Anatomy, Johns Hopkins Medical School, 725 North Wolfe Street, Baltimore, Maryland 21205, USA

G. W. JOURDIAN Rackham Arthritis Research Unit, School of Medicine, University of Michigan, Ann Arbor, Michigan 48109, USA

D. E. KNIGHT Department of Physiology, King's College London, Strand, London WC2R 2LS, UK

S. KORNFELD Division of Hematology-Oncology, Washington University School of Medicine, Box 8125, 660 South Euclid, St Louis, Missouri 63110, USA

J. MELDOLESI Facolta di Medicina e Chirurgia, Istituto di Farmacologia, Universita Degli Studi, Via Vanvitelli 32, 20129 Milano, Italy

I. S. MELLMAN Section of Cell Biology, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut 06510, USA

G. E. PALADE Section of Cell Biology, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut 06510, USA

B. M. F. PEARSE MRC Laboratory of Molecular Biology, Medical Research Council Centre, University Medical School, Hills Road, Cambridge CB2 2QH, UK

M. RAFF Department of Zoology, University College London, Gower Street, London WC1E 6BT, UK

R. RODEWALD Institut de Biochimie, Université de Lausanne, CH-1066 Epalinges, Switzerland. *Permanent address*: Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, Virginia 22901, USA

J. E. ROTHMAN Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305, USA

D. D. SABATINI Department of Cell Biology, New York University Medical Center, New York, NY 10016, USA

Y.-J. SCHNEIDER International Institute of Molecular and Cellular Pathology, Université Catholique de Louvain 7539, 75 avenue Hippocrate, B-1200 Bruxelles, Belgium

W. S. SLY Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305, USA. *Permanent address*: Division of Medical Genetics, Department of Pediatrics, Washington University School of Medicine, St Louis, Missouri 63110, USA

C. C. WIDNELL Department of Anatomy and Cell Biology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, USA

Problems in intracellular membrane traffic

GEORGE E. PALADE

Section of Cell Biology, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut 06510, USA

Abstract Eukaryotic cells operate an extensive and well regulated traffic of membrane-bound vesicles to: (a) transport intracellularly, and eventually discharge by exocytosis, macromolecular products; (b) take up by endocytosis molecules and particles from the environment; (c) transport macromolecules across epithelial barriers; and (d) move membranes from their site of assembly to their final locations. Vesicular transport appears to be the equivalent of a discontinuous circulatory system in which vesicles recycle between the termini of each transport pathway, so that balanced membrane distribution is maintained among cell compartments and the cell's surface. Although the general outline of the process is reasonably clear, much remains to be learned about the number and types of pathways, the types and quantities of membranes, and the rates of vesicular movement. Since each vesicular carrier finds its specific terminus (and fuses with it), vesicular traffic is strictly controlled. By analogy with the control of intracellular protein traffic, it may be assumed that vesicular traffic is regulated by the mutual recognition of protein signals and receptors affixed, in this case, with appropriate asymmetry to the surface of the interacting membranes. Since vesicular transport operates without loss of specific chemistry and function of various cellular membranes, cells can counteract effectively the randomization of membrane proteins and lipids, which becomes possible whenever two membranes establish continuity of their fluid bilayers, and when membrane is removed from one or both termini of a recycling pathway. Specific selection of termini and prevention of randomization among membrane components are major unsolved problems in vesicular transport. Their solution in terms of molecular interactions requires further work.

Membrane specificity

A eukaryotic cell is an extensively compartmented system. Within its boundaries, which are set by the cell's membrane or plasmalemma, it accommodates a relatively large number of intracellular compartments, each of them delimited by its own membrane. At present, we can distinguish in an animal eukaryote at least 10 different types of membrane, each type being

1982 Membrane recycling. Pitman Books Ltd, London (Ciba Foundation symposium 92) p 1–14

identified by its specific structure, chemistry and function, as well as by the characteristic morphology of the compartment it outlines.

Chemical specificity at the subcellular level is clearly expressed by the concept of marker component (or marker enzyme) proposed by de Duve and his collaborators more than 25 years ago (see de Duve 1963). It applied originally to subcellular particles, and it implied that certain molecular components (or activities) are restricted in their distribution to specific types of subcellular structure. The concept can be extended to cellular membranes since many subcellular particles are membrane-bounded compartments, and many markers are intrinsic membrane proteins with well defined enzymic activities. At a general and not particularly stringent level, the marker concept is still valid and useful, but in detail and at critical levels it should be redefined to take into account more recent findings and developments in cell biology, including membrane recycling.

Cellular membranes are diverse in their functions—primarily as a result of specific differences among their protein components—but all have a common structural and biochemical denominator: a bimolecular film of polar lipids—the lipid bilayer (for short)—that functions as a diffusion barrier for hydrophilic solutes between the cell and its environment, as well as between intracellular compartments. The repertoire of membrane lipids is considerably more limited than that of membrane proteins; moreover, differences from one type of membrane to another are essentially quantitative: the same lipid classes are found—at different relative concentrations—in all cellular membranes; (the only exception is diphosphatidylglycerol, restricted in its distribution to the inner mitochondrial membrane).

Membrane fluidity

By now it has been convincingly established that the lipid bilayers of all cellular membranes are fluid at the temperature of the cell's immediate environment (Singer & Nicolson 1972), and that cells in general have the ability to modify the chemistry of their complex lipids so as to maintain the average transition temperature of the bilayers well below ambient temperature (Cronan & Gelmann 1975), whether the ambience is a polar sea, a hot spring or a homeothermic organism.

We now begin to understand why cells insist on maintaining membrane fluidity: fluid bilayers are prerequisites for membrane growth—by expansion of pre-existing membranes—in anticipation of cell division (Palade 1978); and bilayer fluidity is also required for effective membrane fusion–fission without lysis. This type of membrane interaction occurs at the last step in cell division and at the first step in zygote formation. Membrane fusion–fission is

also required in other, 'every-minute' cell activities, such as vesicular transport of macromolecules and particles from one compartment to another, including endocytosis, exocytosis and compensatory membrane recycling. A point that deserves to be stressed is that membrane fusion–fission leads to continuity being established not only between compartments, but also between the fluid bilayers of interacting membranes.

The corollary of membrane fluidity is a rapid diffusion in the plane of the lipid bilayer of both lipid and integral membrane proteins (Schlessinger et al 1977)*, and hence a potential randomization of the chemistry of any membrane as well as of any pair of interacting membranes. And here we encounter a set of opposite requirements, a contradiction in principles, at the basic level of cellular organization. Cells insist on having membranes that are generally fluid and yet chemically specific, irrespective of the frequency and extent of their interactions. Eukaryotic cells have clearly solved—a few billion years ago—the problem posed by such contradictory demands. It is now our turn to solve it again (so to speak), by deciphering the elements of the solution.

Differentiated domains and stabilizing infrastructures

Eukaryotic cells can circumvent randomization and create differentiated domains within the continuity of their plasmalemma and—most probably—of other membranes. The domains are of varied but specific chemistry, transient or durable existence, varied origin and varied sizes (from < 80 nm to > 10 μm diam). Most of those identified at present appear to arise from interactions between integral membrane proteins and an infrastructure that is generated by peripheral membrane proteins which act as selectors and stabilizing agents for the corresponding domain. This stabilizing infrastructure may involve (1) geodetic cages formed by clathrin and associated proteins, as for coated pits (Pearse 1975, 1978); or (2) fibrillar proteins of still unidentified nature (perhaps actin), as for certain discharging secretory granules (Palade 1975); or (3) more extensive structures which, in extreme cases, immobilize practically all integral membrane proteins (Elgsaeter & Branton 1974), as do spectrins, ankyrin and actin in erythrocytes. A specific stabilizing infrastructure is also the formula used by an enveloped virion in selecting out its own glycoproteins (from the plasmalemmal proteins of the

* Coefficients for lateral diffusion (within the plasmalemma) measured by these workers are about 9×10^{-9} cm^2 s^{-1} for lipids and about 2×10^{-10} cm^2 s^{-1} for proteins. Linear approximations of these values are 1 μm s^{-1} for lipids and 1 μm min^{-1} for proteins.

host cell), and in stabilizing the patch of cell membrane that, upon budding, will become the envelope of the virion (Compans & Klenk 1979).

Other stabilizing interactions

In principle, differentiated microdomains could also be created by interactions among integral membrane proteins within the membrane or within the lipid bilayer itself. Many such interactions have been suggested but none has been well documented. Moreover, in multicellular organisms, differentiated microdomains within the plasmalemma of a given cell can be generated by 'in phase' interactions with an adjacent partner cell. In such cases, stability is achieved by interactions within each membrane as well as between membranes, as in 'gap' (or communicating) junctions, or by multiple interactions with stabilizing infrastructures, with homologous molecules within one membrane, and with complementary molecules in the plasmalemma of the partner cells, as in the elements of junctional complexes. Some junctional elements, such as the occluding zonules of epithelial cells, create apparently uninterrupted barriers within the continuity of the plasmalemma and thereby they separate from one another relatively large plasmalemmal domains that face different extracellular compartments and differ significantly in structure, chemistry and function.

Thus, eukaryotic cells have evolved procedures to counteract randomization of membrane proteins and to generate differentiated domains of stabilized chemistry by a variety of interactions. This ability plays an important part in vesicular transport and membrane recycling, since it allows the cell to perform such operations without losing the chemical specificity of the interacting membranes. In fact, some of the differentiated microdomains already mentioned—the coated pits and the stabilized membranes of discharging secretion vacuoles (or granules)—can be recognized at present as termini of certain pathways of vesicular transport.

Vesicular transport

Eukaryotic cells make extensive use of vesicular containers to export—by *exocytosis*—macromolecules and macromolecular complexes into their environment, and to import—by *endocytosis*—macromolecules and particles, together with a variable amount of fluid, from their external medium.

Endocytosis

In endocytosis, vesicles of various sizes and at least four different types (i.e. phagocytic vacuoles, pinocytic vacuoles, plasmalemmal vesicles, or micro-

pinocytic vesicles, and coated vesicles) transport imported molecules and particles from the cell's surface to secondary lysosomes (Silverstein et al 1977), either directly or through one or two intermediary compartments. The membrane of these vesicular carriers is eventually returned to the cell surface so that, in steady state conditions, each compartment or interacting membrane retains a relatively constant surface area. The cells therefore move vesicles along a circuit (the endocytic circuit) in which the inward arc is directly involved in importing matter, whereas the outward arc ensures that membrane is retrieved from either a lysosomal or a pre-lysosomal compartment. The extent to which the membranes of all these carriers are similar to, or different from, one another—and from the membranes of their terminal or intermediary stations—is discussed in this volume primarily by Cohn & Steinman, Mellman, Pearse and Bretscher. Membrane recycling usually refers to the second arc of the circuit and implies that the membranes of the corresponding vesicular carriers are repeatedly reutilized.

Exocytosis and intracellular transport

In *overt* secretion the export of macromolecules involves a series of intracellular compartments, namely the endoplasmic reticulum (ER), the Golgi complex, and secretion vacuoles (or granules) that eventually discharge their contents into the external medium (Palade 1975). Part of the same pathway is used to transport lysosomal hydrolases from the ER to the Golgi complex and eventually to primary and secondary lysosomes in *covert* secretion. The same pathway appears to be involved in the transport of membrane proteins (already assembled into a lipid bilayer) from the ER to the plasmalemma, and the same probably applies for membrane proteins with other destinations, such as Golgi membranes or lysosomal membranes. In such cases, the species of primary interest are the membrane proteins (rather than the content proteins) of the vesicular carriers.

Along the secretory pathway, vesicular transport operates at two junctions, namely between the ER and the Golgi complex (Jamieson & Palade 1967a,b), and between the Golgi complex and the plasmalemma or (for exocrine cells) the specialized luminal domain of the plasmalemma (Palade 1975). Small vesicles of about 50 nm diameter appear to operate at the first junction, and larger vesicles of up to 500 nm diameter, usually designated as secretion vacuoles or granules, are the carriers at the second junction. The sites at which vesicular transport operates along the secretory pathway may, however, be more numerous, since the Golgi complex apparently consists of a series of functionally distinct compartments (as discussed in this volume by Kornfeld et al and Rothman), which may also be connected to one another by vesicular carriers.

In secretory cells, transporting vesicles continuously move macromolecular products from one compartment to another along the secretory pathway. Yet, under steady state conditions, each compartment and each interacting membrane retains a relatively constant surface area, because membrane appears to be continuously recovered by the donor compartment from its receiving partner. In such cells, two circuits can be recognized at present: one between the ER and the Golgi complex, and the other between the Golgi complex and the plasmalemma. At each site, the centrifugal (outward) arc of each circuit carries secretory products, whereas the centripetal (inward) arc is involved in the recovery of container membranes (see Farquhar, this volume).

It can be assumed that vesicular carriers are also involved in the transport of lysosomal hydrolases from the Golgi complex to lysosomes (Friend & Farquhar 1967) and in the transport of pre-assembled membrane proteins from some subcompartment of the Golgi complex to different plasmalemmal domains, and perhaps to other cellular membranes. In certain epithelial cells, vesicular carriers function in the transport of macromolecules from one extracellular compartment to another, across the epithelium, in an operation described as *diacytosis* or *transcytosis*, which appears to be differentially amplified in the vascular endothelium (Palade et al 1979). Diacytosis in the intestinal epithelium is discussed in this volume by Rodewald & Abrahamson (see also p 109-115). Recycling of vesicular containers has also been postulated in diacytosis (Palade et al 1979, Simionescu et al 1981).

A eukaryotic animal cell therefore appears to operate a number of discontinuous circulatory systems, present in all cell types, but individually amplified in some as a result of cell differentiation. What is still uncertain is the extent to which these multiple pathways are interconnected, or use common vesicular carriers or different carriers that move along common arcs. A connection between the endocytic and exocytic pathway has been detected in secretory cells (see Farquhar, this volume) and a link between the transcytotic, endocytic and exocytic pathways apparently exists in intestinal epithelia (see Rodewald & Abrahamson, this volume).

An attempt to rationalize vesicular transport

The existence of distinct compartments connected by shuttling vesicular carriers can be rationalized in terms of a series of well established findings and reasonable postulates. Protein synthesis (for whatever final destination) is essentially centralized in a single cell compartment, i.e. the cytoplasmic matrix or cytosol*; all proteins produced for overt or covert secretion are

* (Only a small fraction ($\leq 2\%$) of the cell's proteins is produced in the mitochondrial matrix by mitochondrial ribosomes.)

translocated across the ER membrane into the cisternal space of the ER, the first compartment of the secretory pathway. A still unknown, but presumably large number of integral membrane proteins, destined for the plasmalemma (and presumably for other cellular membranes), is also inserted into the ER membrane (Blobel et al 1979, Sabatini et al 1982). All these proteins are modified co- or post-translocationally by complex biochemical reactions (e.g. partial proteolysis, glycosylation of a variety of types, sulphation, phosphorylation and amino acid residue modifications) specific for each compartment of the secretory pathway (i.e. ER, Golgi complex and secretion vacuoles). From an initially common duct system, i.e. the ER, content proteins are sorted out into secretory and lysosomal proteins and are eventually separated into distinct and different compartments (secretion vacuoles versus lysosomes). The sorting-out operation is probably more elaborate for membrane proteins, since each of them eventually reaches its proper destination via vesicular carriers that presumably depart from the Golgi complex. In addition to modifications incurred by individual macromolecules, the macromolecular solutes may be extensively concentrated (as in condensing vacuoles and secretion granules), or the properties of their solvent may be changed (as in lysosomes). Each of these numerous operations probably requires a specific environment, and hence a distinct compartment, to which access can be controlled. Vesicular transport may be the way of supplying macromolecular substrates to each of these operations with a minimum of disturbance to the conditions prevailing within each receiving compartment. A continuous conduit with several functionally differentiated segments (in the style of a nephron) may not provide adequate environmental separation for the series of operations mentioned above. Vesicular transport of secretory proteins is known to be vectorial (in the few cases so far studied), the receiving compartment acting as an efficient sink so that backflow or diffusion up the pathway is prevented. A similar performance may be difficult to achieve in a continuous conduit in the absence of rapid flow.

The secretory pathway resembles a modern industrial assembly line, since it is built to perform a series of operations in which each step depends on the completion of the previous one. The cellular 'assembly line' requires, however, a different environment for most—if not each—of these operations, and it therefore takes the form of a series of connectable compartments rather than of a continuous, moving band.

Problems generated by vesicular transport

Many direct observations show that eukaryotic cells have evolved efficient procedures for the control of vesicular transport. Notwithstanding the large

number of diverse carriers, the multiplicity and complexity of circuits, the crowding and the complex geometry of the cell's interior, each carrier appears to recognize its proper destination and to interact with its proper partner, to which it eventually delivers either its content or its membrane. So far no obvious 'mistakes' have been recorded under normal operational conditions.

Traffic control

Vesicular traffic is obviously carefully controlled but the means by which control is achieved is still completely unknown. We can draw an analogy with the extensively studied mechanisms that control the traffic of newly synthesized proteins from the cytosol to a multiplicity of final destinations (Walter et al 1981, Walter & Blobel 1981). Thus, we may assume that the mutual recognition of a signal (affixed to a vesicular carrier) by a signal receptor (borne by the membrane of its compartmental partner) leads to a series of interactions that eventually result in binding, followed—in this case—by membrane fusion–fission between the vesicular carrier and its appropriate receiving compartment. The assumption implies that signals as well as receptors are membrane-bound, that their recognition sites are exposed on the cytoplasmic aspect of the corresponding membranes, and that each circuit is provided with its specific set of pilots (or signals) and ports of entry (or signal receptors).

Because many homologous compartments, such as mitochondria, lysosomes and secretion granules, often undergo fusion–fission (restricted specifically to members of the same set of compartments), it can be further postulated that in vesicular transport each interacting membrane carries both the signal (**S**) and its receptor (**R**) as reciprocal doublets, so that **S–R** recognizes and is recognized by **R–S**.

Control of membrane specificity

The second major problem connected with vesicular transport concerns the retention of chemical specificity by all the membranes involved in a given circuit, i.e. usually the membranes of the two compartments that function as donor and receiving termini and the membrane of the shuttling vesicular connector (Palade 1976).

Unless prevented by appropriate means, loss of specificity becomes unavoidable at two different steps for two different reasons. (1) In the process of fusion–fission of the membrane of a vesicular carrier with that of a receiving compartment, continuity between the two corresponding bilayers is estab-

lished and rapid lateral diffusion (in the plane of the fused membranes) is to be expected. It should lead to intermixing and eventual randomization of the components of the interacting membranes, unless one or the other membrane (or both) has its chemistry stabilized for the duration of bilayer continuity. (2) When excess membrane is removed from the receiving compartment to be returned to the donor compartment, so as to maintain the balanced membrane distribution that characterizes the steady-state operation of the circuit, randomization again becomes possible (this time for a different reason), unless prevented. If the cell removes from the receiving terminus, and returns to the donor terminus, a membrane patch equivalent in surface area, but not in chemistry, to the membrane of the vesicular carrier already received, balanced distribution of membranes can be maintained but randomization of chemistry and function will unavoidably ensue throughout the circuit. To prevent randomization, removal of excess membrane must follow a non-random procedure which ensures that the membrane removed is the same in surface area and chemistry as the membrane received (Palade 1976). But for a removal of this type to be possible, the membrane of the incoming vesicle must be stabilized throughout its residence in the membrane of the receiving terminus. Stabilization of the incoming vesicle membrane could be achieved by some of the means already discussed, in conjunction with differentiated microdomains within the plasmalemma. In fact the same means of stabilization (e.g. interactions with a stabilizing infrastructure) could prevent randomization at the time of bilayer fusion and make possible the subsequent non-random removal of the carrier membrane.

A priori, stabilization would be required at both the donor and receiving termini if the vesicular carrier had a membrane of specific chemistry distinct from that of each of the termini. But stabilization would be needed at one terminus only if the membrane of the carrier were identical to that of the other terminus. Some evidence already suggests that cells use both alternatives. The membranes of some endocytic vacuoles or vesicles appear to be qualitatively similar to the plasmalemma (see Mellman, this volume), and vesicles that transport secretory proteins and lipoproteins from the ER to the Golgi complex in hepatocytes appear to have a number of antigens and enzymic activities in common with the ER membrane (Ito & Palade 1978). But, in receptor-mediated endocytosis a special (coated) vesicle, distinct from the rest of the plasmalemma is involved. (Pearse & Bretscher 1981, Goldstein et al, this volume). Secretion granule membranes, isolated from the pancreas, parotid (Castle et al 1975) or adrenal (Winkler 1971), appear to be much simpler membranes than the plasmalemma, and possibly the Golgi membranes, but in all these examples the evidence is incomplete, in that a reliable comparison of the membrane chemistry of the carrier and the relevant domains at both termini is not yet on record.

In principle, cells have the option of stabilizing either the membrane of the vesicular carriers (as discussed above) or the membrane of one (or both) termini; and the available evidence suggests that they use both options. Vascular endothelial cells are remarkable for the diversity and surface density of their differentiated plasmalemmal microdomains (Simionescu et al 1981). Plasmalemmal vesicles involved in diacytosis (or transcytosis) are part of the local repertoire of differentiated microdomains. Studies indicate so far that this vesicle membrane is different from the plasmalemma proper and that the latter (rather than the vesicles themselves) is backed by a fibrillar infrastructure that presumably has a stabilizing function. A similar situation is encountered in neuromuscular junctions (Ceccarelli et al 1979) and presumably in synapses. Such an infrastructure may also define docking sites for incoming and departing vesicles, in addition to stabilizing the chemistry of the plasmalemma proper. The same alternative may apply for the membranes of Golgi cisternae which are kept in situ in characteristic alignment*. Interactions of this type may explain why Golgi cisternae can be isolated as intact stacks by certain fractionation procedures (see Farquhar & Palade 1981).

A second look at stabilizing infrastructures

At present the outstanding example of stabilizing infrastructure is provided by the budding of enveloped virions. A structure that consists of matrix or nucleocapsid proteins is found immediately under the plasmalemma in phase with a patch of membrane within which viral glycoproteins are concentrated, whereas host membrane proteins are usually excluded (Compans & Klenk 1979). Viral glycoproteins are transmembrane proteins with a relatively small endodomain. It is assumed, but not proven, that the budding process is controlled (in part) by interactions between these endodomains and elements of the stabilizing infrastructure. These interactions probably ensure selection of viral glycoproteins, and exclusion of host proteins; in addition, they apparently control the direction of budding and the size and shape of viral particles (see Rindler et al, this volume).

Except for an inverted geometry—'budding' proceeds towards the cytosol rather than towards the external medium—the geodetic cages formed by clathrin (and associated proteins) have many properties in common with viral matrix and nucleocapsid proteins. The cage may act as both selector and stabilizer, and may also control the direction of 'budding' as well as the size and shape of the coated vesicles finally produced. Selection of membrane

* (Fibrillar structures have been detected [see Farquhar & Palade 1981] in between Golgi cisternae in a few cases.)

proteins undoubtedly occurs but the degree of selection—and conversely of exclusion—is still unknown for proteins as well as for lipids (Pearse & Bretscher 1981; Pearse, this volume; Bretscher, this volume). The stability of the interactions between the clathrin infrastructure and the integral proteins of the membrane appears to vary. In the rather well documented case of coated pits involved in receptor-mediated endocytosis, for instance, some receptors (such as LDL receptors) appear to be permanently located in the pits, while others (such as the receptors for epidermal growth factor, insulin, transferrin, asialoglycoproteins and different types of enveloped virus) are collected into coated pits only after interacting with their respective ligands before being endocytosed (see contributions by Goldstein et al, Cuatrecasas, Helenius and Hubbard in this volume).

The stabilizing infrastructures of animal eukaryotes thus seem to be more versatile than their viral counterparts. They may allow the membrane components of an incoming vesicle to disperse into the membrane matrix of a terminus, and they may depend on changes in affinity (induced by ligand binding or clustering) in order to recapture these components before non-random removal from that terminus is effected, as seems to happen for many plasmalemmal receptors.

Although coated pits and coated vesicles have attracted attention mostly as agents involved in the early steps of receptor-mediated endocytosis, it should be stressed that similar structures are found to be associated with many (but not all) intracellular compartments. Equivalents of coated pits are detected on endosomes (see Helenius, this volume), Golgi cisternae, Golgi condensing vacuoles (Jamieson & Palade 1967a,b, Farquhar & Palade 1981) and, in a simplified version, in the transitional elements of the ER (Palade 1975, Palade & Fletcher 1977). Hence, clathrin and associated proteins may function as selectors and stabilizers of vesicular carrier membranes at the termini of a number of vesicular transport circuits.

A certain amount of information is currently available about clathrin and its associated proteins (Pearse & Bretscher 1981, Pearse 1982) as well as about conditions involved in their co-polymerization into geodetic cages (Unanue et al 1981). But, except for the presence of the receptors already mentioned, we know much less about the membrane components of coated pits and coated vesicles, and we depend exclusively on assumptions and speculations when considering the nature of the transmembrane interactions involved in the assembly of these structures.

As already mentioned, other types of stabilizing infrastructure and interaction should be considered in relation to the control of membrane specificity in vesicular transport.

A wide-open question concerns the mechanisms operating in situ to assemble and dismantle these stabilizing infrastructures and the possibility

that elements of the locomotor apparatus of the cell play some part in the movement of coated vesicles to their appropriate destinations.

Perspectives

Membrane recycling, viewed as an integral part of vesicular transport, will substantially affect our thinking and our experimental approaches over the next few years. We can expect to see different types of cytoplasmic free vesicle and coated vesicle, similar perhaps in appearance but different in membrane chemistry, each type representing a specific carrier for one of the five or six circuits that we have identified so far in animal cells. Lumping together all smooth vesicles under the term smooth ER (and, hence, smooth microsomes) and all coated vesicles under another single heading will become a thing of the past. Transient residence of stabilized vesicular carriers in one or both termini of a given circuit may lead to a non-coincidence of structural and chemical boundaries among cellular compartments. We assume, for instance, that a Golgi cisterna is a typical, unitary, structural element of the Golgi complex; chemically, it may turn out to be a mosaic. The same may apply to the plasmalemma: a coated pit may be a carrier vesicle in transient residence in the cell membrane; chemically that pit may belong to a different intracellular membrane system*. In other words, we may be obliged to reorder our traditional categories of cellular compartments and membranes.

For the same reasons, the marker concept—used in guiding present-day fractionation procedures and in interpreting their results—will have to be redefined, because the transient residence of a vesicular carrier of specific chemistry in the membrane of a terminal compartment of different chemistry may introduce an apparently 'extraneous' marker in the cell fraction that represents the terminal compartment. That 'extraneous' marker will be found there on account of function, not necessarily on account of particulate contamination, as we usually assume at present. In addition, we shall need more refined separation procedures for subcellular components, based whenever possible on specific chemical interactions (see Ito & Palade 1978, Merisko et al 1982) rather than on general non-specific physical parameters, to be able to sort out the new categories of vesicle involved in vesicular transport and recycling.

An awareness of the diversity and extent of vesicular transport should prompt us to work towards a better understanding of the molecular mechan-

* A different interpretation is proposed, however, by Pastan and his collaborators (see Willingham & Pastan 1980); they assume that coated pits are stable plasmalemmal structures which generate uncoated vesicles that transport ligands to 'receptosomes'.

isms that control vesicular traffic and membrane specificity, not to mention those involved in membrane fusion–fission.

In lieu of conclusion

This paper is primarily a logical construct based on premises provided by established facts and currently recognized limitations within which eukaryotic animal cells must function. In the ultimate analysis, these facts and limitations indicate that membrane traffic and membrane specificity must be controlled. Yet this construct extends far beyond established facts: it hangs in the unknown, supported only by untested logical extrapolations. Therefore, some of its aspects may prove to be wrong, but others may have the merit of being reasonable working hypotheses that, in due time, could be put to experimental test.

REFERENCES

Blobel G, Walter P, Chang CH, Goldman BM, Erickson AH, Lingappa VR 1979 Translocation of proteins across membranes: the signal hypothesis and beyond. Symp Soc Exp Biol 33:9-36

Castle JD, Jamieson JD, Palade GE 1975 Secretion granules of the rabbit parotid gland. Isolation, subfractionation and characterization of the membrane and content subfractions. J Cell Biol 64:182-210

Ceccarelli B, Grohoraz F, Hurlbut WP 1979 Freeze fracture studies of frog neuromuscular junctions during intense release of neurotransmitter. J Cell Biol 81:163-177

Compans RW, Klenk H-D 1979 Viral membranes. In: Fraenkelconrat H, Wagner RR (eds) Comprehensive Virology. Plenum Press, New York, vol 13:293-407

Cronan JE, Gelmann EP 1975 Physical properties of membrane lipids: biological relevance and regulation. Bacteriol Rev 39:232-256

de Duve C 1963 The separation and characterization of subcellular particles. Harvey Lect 59:49-87

Elgsaeter A, Branton D 1974 Intramembrane particle aggregation in erythrocyte ghosts. I: The effects of protein removal. J Cell Biol 63:1018-1030

Farquhar MG, Palade GE 1981 The Golgi apparatus (complex)-(1954–1981)-from artifact to center stage. J Cell Biol 91:77s-103s

Friend DS, Farquhar MG 1967 Functions of coated vesicles during protein absorption in the rat vas deferens. J Cell Biol 35:357-376

Ito A, Palade GE 1978 Presence of NADPH-cytochrome P450 reductase in rat liver Golgi membranes: evidence obtained by an immunoadsorption method. J Cell Biol 79:590-597

Jamieson JD, Palade GE 1967a Intracellular transport of secretory proteins in the pancreatic exocrine cell. I: Role of the peripheral elements of the Golgi complex. J Cell Biol 34:577-596

Jamieson JD, Palade GE 1967b Intracellular transport of secretory proteins in the pancreatic exocrine cell. II: Transport to condensing vacuoles and zymogen granules. J Cell Biol 34:597-615

Merisko EM, Farquhar MG, Palade GE 1982 Coated vesicle isolation by immunoadsorption on *Staphylococcus aureus* cells. J Cell Biol 92:846-857

Palade GE 1975 Intracellular aspects of protein secretion. Science (Wash DC) 189:347-358

Palade GE 1976 Interactions among cellular membranes; problems and perspectives. In: Passino R (ed) Biological and artificial membranes and desalination of water: proceedings. Elsevier, Amsterdam (Pontif Acad Sci Scr Varia) vol 40:85-97

Palade GE 1978 Membrane biogenesis. In: Solomon AK, Karnovsky M (eds) Molecular specialization and symmetry in membrane function. Harvard University Press, Cambridge, p 3-30

Palade GE, Fletcher M 1977 Reversible changes in the morphology of the Golgi complex induced by the arrest of secretory transport. J Cell Biol 75:371a

Palade GE, Simionescu M, Simionescu N 1979 Structural aspects of the permeability of the microvascular endothelium. Act Physiol Scand Suppl 463:11-32

Pearse BM 1975 Coated vesicles from pig brain: purification and chemical characterization. J Mol Biol 97:93-98

Pearse BM 1978 On the structural and functional components of coated vesicles. J Mol Biol 126:803-812

Pearse BMF 1982 Coated vesicles from human placenta carry ferritin, transferrin and immunoglobulin G. Proc Natl Acad Sci USA 79:451-455

Pearse BMF, Bretscher MS 1981 Membrane recycling by coated vesicles. Annu Rev Biochem 50:85-101

Sabatini DD, Kreibich G, Morimoto T, Adesnik M 1982 Mechanisms for the incorporation of proteins in membranes and organelles. J Cell Biol 92:1-22

Schlessinger J, Axelrod D, Koppel DE, Webb WW, Elson EL 1977 Lateral transport of a lipid probe and labeled proteins on a cell membrane. Science (Wash DC) 195:307-309

Silverstein SC, Steinman RM, Cohn ZA 1977 Endocytosis. Annu Rev Biochem 46:669-721

Simionescu N, Simionescu M, Palade GE 1981 Differentiated microdomains on the luminal side of the capillary endothelium. I: Preferential distribution of anionic sites. J Cell Biol 90:605-613

Singer SJ, Nicolson GL 1972 The fluid mosaic model of the structure of cell membranes. Science (Wash DC) 175:720-731

Unanue ER, Ungewickell E, Branton D 1981 The binding of clathrin triskelions to membranes from coated vesicles. Cell 26:439-446

Walter P, Blobel G 1981 Translocation of proteins across the endoplasmic reticulum. II: Signal recognition protein (SRP) mediates the selective binding to microsomal membranes of in vitro assembled polysomes synthesizing secretory proteins. J Cell Biol 91:557-561

Walter P, Ibrahimi I, Blobel G 1981 Translocation of proteins across the endoplasmic reticulum. I: Signal recognition protein (SRP) binds to in vitro assembled polysomes synthesizing secretory proteins. J Cell Biol 91:545-556

Willingham MC, Pastan I 1980 The receptosome: an intermediate organelle of receptor-mediated endocytosis in cultured fibroblasts. Cell 21:67-77

Winkler H 1976 The composition of adrenal chromaffin granules: an assessment of controversial results. Neuroscience 1:65-80

Phagocytosis and fluid-phase pinocytosis

ZANVIL A. COHN and RALPH M. STEINMAN

Cellular Physiology and Immunology Laboratory, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

Abstract The generation, flow, directionality and fusion of phagocytic and fluid-phase pinocytic vesicles in cultured macrophages and fibroblasts are reviewed. Specific plasma membrane (PM) receptors, receptor mobility, contractile cytoplasmic elements and lipid composition of the PM serve to regulate the flow of large phagosomes into the perinuclear zone. Fluid-phase vesicles are constitutively generated and carry large quantities of PM, fluid and solutes into the cytoplasm. Quantitative information is cited on the rates of vesicular generation, fusion with other members of the vacuolar system, fluid and solute uptake, and digestion and solute release. The nature and composition of fluid-phase vesicles, phagocytic vacuoles and PM are compared. Once interiorized, PM and its component polypeptides rapidly cycle back to the cell surface. The flow rates of both the centrifugal and the centripetal compartments as well as the fate of a minor degradation pool are illustrated and compared to the turnover of individual membrane polypeptides. Implications of membrane flow for cell shape, motility and new PM insertion are discussed.

We wish to discuss the bi-directional flow of solutes and membrane into and out of cells in culture. For this purpose we must first delineate the cellular compartments involved, the nature of the solutes, the composition of membranes and the motive forces used by the macrophage and fibroblast to interiorize membrane, to move the newly formed endocytic vesicle (endosome) into the cytoplasm, to fuse it with other members of the vacuolar apparatus and, finally, to dispose of solutes and to recycle membrane constituents. For this purpose we shall use results from structural, cytochemical and biochemical techniques to arrive at quantitative estimates.

Vacuolar apparatus

Most of the information that we have accrued over the past decade concerns

1982 Membrane recycling. Pitman Books Ltd, London (Ciba Foundation symposium 92) p 15–34

the macrophage, which will serve as the focus of our paper, but we shall also present corroborative and comparative information relating to the fibroblast.

The macrophage is a rather plastic cell which, through its endocytic activity, can modify the relative sizes of its vacuolar compartments. In steady-state conditions of culture in 10–20% fetal bovine serum, the mouse peritoneal cell maintains a stable surface area as it remains adherent to substrates of plastic or glass, where there is an intermediate layer of serum and cellular-derived proteins on which the macrophage resides. In addition to the plasma or limiting membrane, the vacuolar apparatus consists of the variety of vesicles and vacuoles in transit through the cytoplasm, many of which are of endocytic origin. About 20% of these are visible by phase-contrast optics in the living cell (Cohn 1966). Apparently, not all vesicles seen in the living cell flow inwards, but follow a centrifugal path towards the plasma membrane.

We must also consider a third form of vesicle in our discussion of the vacuolar apparatus. This is the primary lysosome derived from the Golgi–endoplasmic reticulum complex (GERL). These vesicles are usually indistinguishable from small pinocytic vesicles except for their more central distribution and their content of acid hydrolases (Cohn et al 1966). To our knowledge the number and flow rates of primary lysosomes have not been quantitated in any cell type. Although the major flow of vesicles bounded by membranes and originating from GERL is directed toward the lysosomal compartment, small amounts of cationized ferritin, as in other cell types, have been reported to accumulate in presumed Golgi saccules.

Table 1 gives the major dimension of the components of the macrophage vacuolar apparatus. The plasma membrane area, 825 μm^2, of macrophages as

TABLE 1 Steady-state dimensions of pinocytic vesicles and secondary lysosomes in macrophages

	Pinocytic vesicles	*Secondary lysosomes*
Number per cell	240	1100
Diameter (μm)	0.202	0.136
Area (μm^2)	0.196	0.109
Volume (μm^3)	0.0143	0.0074
% Total volume	2.5	2.5
% Total surface area	12.5	18.0
Total MØ volume = 395 (μm^3)		
Total plasma membrane surface area = 825 (μm^2)		

derived by stereological techniques (Steinman et al 1976), is about 3.3-fold higher than one would have estimated from calculations based on a simple sphere. The difference in surface area is the result of many folds of redundant plasma membrane (PM), particularly in circulating macrophages or in cells washed from the peritoneal cavity. As the cell adheres to substrates, this

preformed membrane is utilized for the spreading process (Bianco et al 1976). Similarly, if large numbers of non-digestible polystyrene latex particles are ingested, thus trapping PM around them, the cell surface becomes progressively smoother as the number of ingested particles increases, and before the overall cell shape changes markedly. Eventually, if enough particles are ingested, cell processes are retracted and rounding of the cell takes place.

The two other significant members of the vacuolar system are largely derived from the PM: the populations of lucent pinocytic vesicles and dense secondary lysosomes. Table 1 gives surface area and volume measurements of macrophages based on stereological techniques and on the use of horseradish peroxidase (HRP) as the fluid-phase solute (Steinman et al 1976). At any given time about 250 vesicles are present in the macrophage and are in transit to the centrosphere region. Fusion occurs frequently, resulting in larger vesicles. The mean pinocytic vesicle diameter of 0.202 μm was measured by using short HRP pulses before extensive fusions. Pinocytic vesicles are present throughout the cytoplasm and originate in an apparently random fashion from all segments of the membrane. The average secondary lysosome is somewhat smaller in size although this compartment may show a wide range of sizes and composition. In these steady-state conditions the combined volumes of the pinocytic vesicle and secondary lysosome compartments is 5.0% of the total macrophage volume whereas their combined fractional surface area is 30% of macrophage surface area. The size of the secondary lysosome compartment can easily be altered, and the storage of a wide variety of poorly or non-digestible products leads to more than a two-fold increase in total lysosomal volume (Cohn & Ehrenreich 1969).

The stereological information obtained by grid intersections and by point counting from sectioned material is in general agreement with results from compartmental analysis by other techniques. For example, Werb & Cohn (1971a), in studies of membrane exchange of cholesterol noted that 70% of the cholesterol was in a rapidly exchanging compartment and 30% was in a more slowly exchanging pool. These calculations, based on a two-compartment model, were extended to show that the larger pool represented PM and the smaller one secondary lysosomes (Werb & Cohn 1971b). In contrast, alveolar macrophages, with their large number of lysosomes, had a slow pool which was enlarged to 54% of the total.

Analogous information was obtained by Edelson & Cohn (1976a) when they examined the cellular compartments of 5′-nucleotidase (EC 3.1.3.5) activity in peritoneal macrophages. This is an ectoenzyme expressed on the cell surface and inhibited with the diazonium salt of sulphanilic acid (DASA)—an impermeant reagent. When peritoneal macrophages were exposed to appropriate concentrations of DASA, about 70% of the 5′-nucleotidase activity was lost, leaving the remaining 30% in an intracellular

pool (Edelson & Cohn 1976b). The majority of the intracellular enzyme was considered to be in an endocytic compartment and was probably distributed in both pinocytic vesicles and secondary lysosomes. Immediately after the uptake of a load of latex particles this pool expanded by as much as three-fold. Thereafter, the intracellular pool of enzyme surrounding the latex particles was inactivated within a few hours. This is in keeping with the rapid inactivation of 5′-nucleotidase when PM is interiorized about latex particles (Werb & Cohn 1972).

Membrane interiorization—quantitative aspects

We shall review and compare here the uptake of soluble and particulate molecules by macrophage, although a discussion of the many factors that influence these two forms of endocytosis is beyond our present scope. Table 2

TABLE 2 Comparison of fluid-phase pinocytosis with phagocytosis in macrophages

	Pinocytosis	*Phagocytosis*
Vesicle diameter (μm)	0.1–1.0 (mean 0.2)	approx 1–>10
Continuous process	Yes	No
Activation energy (kcal mol^{-1})	18	54
19:0 enriched cell[a]	17	90
Temperature range (°C)	2–>38	17–20
Inhibited by cytochalasin	No	Yes
Vesicle fusion inhibited by concanavalin A	Yes	No
Vesicle fusion inhibited by macroanions	No	Yes

[a] 19 carbons: 0 unsaturated bonds

lists some of the more obvious differences between fluid-phase pinocytosis and phagocytosis. Perhaps the most significant is the continuous and constitutive nature of pinocytosis. Pinocytic vesicle formation is set at a fairly constant rate, and we are not aware of any natural inducers in the extracellular environment. Nevertheless, the addition of certain lectins, (e.g. Concanavalin A) will lead to a short-lived increase in solute uptake (Edelson & Cohn 1974a,b). In contrast, the uptake of particulates is a discontinuous process in which the solid is surrounded by a tightly fitting sleeve of membrane, the size of which is governed by particle size. According to the temperature coefficients and calculated activation energies, the Fc receptor-mediated uptake of erythrocytes requires more energy than does the pinocytosis of HRP. Interestingly, manipulations that decrease the fluidity of the PM, such as its