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Chapter 6

Protein Sorting in the Secretory Pathway

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I. INTRODUCTION

From primary and secondary biosynthetic sites in the cytosol and mitochondrial matrix, respectively, proteins and lipids are distributed to more than 30 final destinations in membranes or membrane-bound spaces, where they carry out their programmed function. Work in recent years has started to elucidate how the cell manages to perform this considerable sorting task. Thirty years after Porter *et al.* (1945) discovered the endoplasmic reticulum (ER)—the main avenue for the transport of proteins and lipids in eukaryotic cells (Jamieson and Palade, 1971; Palade, 1975)—the signal hypothesis (Blobel and Sabatini, 1971; Blobel and Dobberstein, 1975a,b) provided the first molecular explanation for the sorting of secretory proteins into the ER lumen. The rate of progress in the last 8 years has been exponential. Palade's suggestion (1975) that the secretory pathway could be utilized to transport molecules destined to reside in the various segments of the pathway itself or in branches of it (such as membrane glycoproteins of the ER, lysosomes, and plasma membrane) has been widely confirmed. Considerable information has been gathered on the intracellular processing of these proteins, particularly on their glycosylation, on the role of signal sequences in protein translocation, and on how hydrolases are imported into lysosomes. The transport of proteins into mitochondria and chloroplasts is now much better understood.

In spite of these advances, our knowledge of the precise mechanisms determining the final location of cellular proteins and lipids is still very scant. The initial hope of finding just a few very general mechanisms has been replaced by more eclectic expectations, as new exceptions to "rules" are described. The development of new model systems, our recently acquired ability to modify genes and reintroduce and express them in eukaryotic cells, and more powerful and sensitive immunolocalization techniques are expected to enable us to provide answers to some of the questions.

In this article we will update the information available on the sorting of molecules in the secretory pathway. Rather than extensively reviewing each subject, we refer the reader to some excellent reviews recently published whenever needed. We have concentrated on a few areas which, we feel, will provide important information in the near future.

II. MOLECULAR SORTING: DEFINITIONS AND FACTORS INVOLVED

Molecular sorting is defined in this article, in its most general sense, as the sum of the mechanisms that determine the distribution of a given molecule from its site of synthesis to its site of function in the cell. Because most of the work in this regard has been centered on protein sorting, we deal mostly with this aspect.

The final site of residence of a protein in a eukaryotic cell is determined by a combination of various factors, acting in concert: site of synthesis; sorting signals or "zip codes;" signal recognition or decoding mechanisms; cotranslational or posttranslational mechanisms for translocation across membranes; specific fusion–fission interactions between intracellular vesicular compartments; and restrictions to the lateral mobility in the plane of the bilayer.

Very little is known about how the differences in lipid compositions of the various cell membranes are generated or about what mechanisms are responsible for their characteristic asymmetric distribution in different halves of the bilayer.

A. Site of Synthesis

Except for a small percentage of mitochondrial and chloroplast polypeptides which are coded by local DNA and synthesized within these organelles, all other cell proteins have a major site of synthesis: the cytosolic polysomes, either free in the cytoplasmic matrix or bound to the membrane of the endoplasmic reticulum. Two principal sites of synthesis also exist for the other major component of membranes, the lipids; these sites are the mitochondria (minor site, mostly producer of cardiolipin) and the ER (major producer of phospholipids and cholesterol) (van Golde *et al.*, 1974; Jelsema and Morre, 1978; Op den Kamp, 1979; Bell and Coleman, 1980). Addition of carbohydrates to glycoproteins and glycolipids also occurs in two major stages: core glycosylation in the ER followed by trimming of this core and addition of branch carbohydrates in the ER and Golgi apparatus (Parodi and Leloir, 1979; Lennarz, 1980; Hubbard and Ivatt, 1981).

B. Sorting Signals or "Zip Codes," Addressing Signals

From the two major sites of synthesis, proteins, glycoproteins, and lipids are distributed to more than 30 membranes or membrane-bound organellar spaces, each of them with a very characteristic composition. Progress in recent years, in particular the confirmation of the "signal hypothesis" (Blobel and Sabatini, 1971; Blobel and Dobberstein, 1975a,b), has substantiated the belief that specific structural features in polypeptides, encoded in their mRNA, guide their sub-cellular distribution. Furthermore, proteins, lipids, and carbohydrates have very defined orientations with respect to the plane of the bilayer, a characteristic which implies that the sorting mechanisms must work in close collaboration with systems to translocate the molecules across the bilayer.

At the moment of synthesis, the cell makes an initial decision on the destination of the proteins based on the possession, by some of them, of specific structural features or "primary addressing signals" (Table I). Secretory proteins and transmembrane or luminal glycoproteins destined to go to the various seg-

TABLE I
MECHANISMS FOR ADDRESSING PROTEINS TO ORGANELLES

Protein destination	Primary addressing signals	Secondary addressing signals	Ref. ^a
<i>Cotranslational insertion</i>			
Secretory proteins	Signal sequences	Probably none	1-5
Integral membrane glycoproteins of			
ER, Golgi, lysosomes, peroxisomes	Signal sequences ?	?	1-8
Plasma membrane	Signal sequences	?	1-5,9
Integral proteins on cytoplasmic side of ER	Insertion signals	?	3,9,14
Luminal proteins of ER, Golgi	Signal sequences ?	?	1-5
Lysosomal hydrolases	Signal sequences	Mannose 6-P	10,11
<i>Posttranslational insertion</i>			
Peripheral and integral proteins on cytoplasmic side of all organelles	Binding sites for integral or peripheral proteins, insertion signals	Probably none	4,9,12,14
Mitochondrial proteins (inner or outer membrane, 2 spaces)	Extra peptides, precursor polypeptides or internal sequences	?	13,14
Chloroplast proteins (inner, outer, thylakoid membranes, 3 spaces)	Extra peptides or internal sequences	?	13,14
Luminal peroxisomal proteins	Internal sequences	?	2,4,6,8

^a Key to references: (1) Blobel *et al.* (1979); (2) Blobel (1980); (3) Kreil (1981); (4) Sabatini *et al.* (1982); (5) Lusis and Swank (1980); (6) Novikoff (1976); (7) Kreibich *et al.* (1978a-c); (8) Lazarow (1980); (9) Lodish *et al.* (1981); (10) Strawser and Touster (1980); (11) Sly (1982); (12) Branton *et al.* (1981); (13) Chua and Schmidt (1979); (14) Poyton (1983).

ments of the secretory pathway (ER, Golgi, plasma membrane), or to branches of it (lysosomes), are *cotranslationally* translocated through the membrane of the rough ER. The structural information in these proteins, or primary addressing signal, is the signal sequence, which is a stretch of 15-30 hydrophobic amino acids, is usually located at the amino terminal end, and is cleaved cotranslationally by a signal peptidase. As predicted by Blobel and Sabatini (1971), the first evidence for its existence came from *in vitro* translation experiments by Milstein *et al.* (1972), who showed that immunoglobulin light chains exhibited extra amino acids at the amino-terminal end when synthesized from mRNA in a reticulocyte lysate in the absence, but not in the presence, of microsomal mem-

branes. Blobel and Dobberstein (1975a,b) obtained the crucial experimental evidence that led to the formulation of the signal hypothesis and the concept of cotranslational segregation. They found (1) synthesis of a large precursor in a cell-free translation system programmed with Ig light chain mRNA, and (2) segregation of normal-size Ig light chains in the microsomal lumen when membranes were added before the start, but not after the completion, of protein synthesis. Subsequently, the existence of precursors was demonstrated for a wide variety of secretory (Blobel *et al.*, 1979) and membrane-bound (Lodish *et al.*, 1981; Sabatini *et al.*, 1982) polypeptides. Sequence information for many of them has been obtained by protein sequencing or, more frequently in recent years, from sequencing cDNA or cloned genes. A discussion of the structure of the signal sequences and their role in protein translocation can be found in the first four articles of this volume. Much of the direct evidence for this role comes from work in prokaryotic systems (reviewed by Davis and Tai, 1980; Emr *et al.*, 1980; Kreil, 1981; see also articles by Duffaud *et al.* and Bankaitis *et al.*, this volume).

Cotranslationally operating hydrophobic sequences which are permanent features of the protein participate in the insertion of cytochrome P-450 and in the translocation across the ER membrane of influenza virus neuraminidase (Bar-Nun *et al.*, 1980; Blok *et al.*, 1982).

Transient amino-terminal sequences are apparently also responsible for the transfer of several proteins into mitochondria and chloroplasts (Chua and Schmidt, 1979; Poyton, 1983; and article by Reid, this volume). The function of this second type of primary addressing signals is different, however, from that of the signal sequences, since these proteins are synthesized by free polysomes and translocated *posttranslationally*.

Other primary addressing signals operating via posttranslational translocation mechanisms are involved in the distribution of peroxisomal and nuclear matrix proteins, which are also synthesized in free cytoplasmic polysomes (Table I). Free polysomes also participate in the synthesis of proteins associated with the cytoplasmic domain of several organelles, such as cytochrome b_5 and NADH-cytochrome b_5 reductase (Borgese and Gaetani, 1980; Rachubinski *et al.*, 1980). Proteins that remain in the cytosol after synthesis by free polysomes presumably lack organellar addressing signals.

In addition to the primary addressing signals mentioned above, other features in the polypeptide chain are thought to be responsible for the particular distribution of proteins within organelles or chains of interconnected organelles (such as those in the secretory pathway). These secondary "sorting signals," or "addressing signals," may also be added co- or posttranslationally to the protein but, of course, the type or the degree of the modification is ultimately determined by structural information in the peptide chain. For example, only proteins which expose in the lumen of the ER Asn-X-Ser(Thr) groups will be core glycosylated;

additional information is required to determine whether that core will be processed to simple or complex residues or to terminal mannose 6-phosphate groups that will target the protein to the lysosomes (Hubbard and Ivatt, 1981; Sly, 1982; Pollack and Atkinson, 1983).

The mannose 6-phosphate group of lysosomal proteins is the only well-characterized "secondary addressing signal." Like secretory proteins, lysosomal hydrolases are synthesized as larger precursors, inserted into the ER lumen via transient signal sequences, and glycosylated by transfer of a mannose-rich core oligosaccharide (see Sly, 1982). This core is processed differently, however, in lysosomal proteins: the action of two enzymes in the Golgi apparatus results in the exposure of several mannose 6-phosphate residues, responsible for the targeting to their final destination (see Section III,D).

C. Signal Recognition or Decoding Mechanisms

The sorting signals must be read and interpreted by specific decoding mechanisms. Advances have been made in recent years in identifying the decoding mechanism for the signal sequence (see Section IV,A,1). A receptor for the mannose 6-P groups of lysosomal proteins—located on the cell surface and, most crucially, in the Golgi apparatus—appears to play a role in the transport to lysosomes (Sly, 1982; see Section III,D). No information is available yet on the decoding systems for the signals of other organellar proteins.

D. Cotranslational and Posttranslational Mechanisms for Translocation across Membranes

Proteins which have hydrophilic segments exposed on the noncytoplasmic or "ecto" domain of membranes require special translocation systems. It is now clear that proteins are translocated across membranes using either co- or post-translational mechanisms but very little is known about the events taking place at the molecular level. Cotranslational translocation is the preferred procedure for exported proteins and transmembrane glycoproteins; it appears to be a very useful method to prevent the accumulation of potentially dangerous secretory toxins in the cytosol. As mentioned, posttranslational translocation has been shown to occur for mitochondrial and chloroplast proteins and peroxisomal enzymes (Dobberstein *et al.*, 1977; Goldman and Blobel, 1978; Chua and Schmidt, 1979; Poyton, 1983).

An interesting example of posttranslational translocation is the entrance of bacterial and plant toxins into cells (Pappenheimer, 1978). These toxins (such as diphtheria and tetanus toxin and ricin) possess a domain that recognizes specific

receptors in the plasma membrane and mediates translocation across the bilayer and a second domain responsible for the toxicity. Membrane penetration is triggered by a slightly acidic pH; it has been shown that these toxins enter the cell via receptor-mediated endocytosis and reach the cytoplasm through the membrane of prelysosomal vesicles (or "endosomes") with acid pH contents (Donovan *et al.*, 1981; Boquet and Duflot, 1982). Similar mechanisms appear to be responsible for the fusogenic properties of various viral envelope glycoproteins (see White *et al.*, 1983) and the penetration of enveloped RNA viruses into cells (Marsh *et al.*, 1983).

E. Specific Fusion–Fission Interactions between Intracellular Vesicular Compartments

Work during recent years has clearly demonstrated that intracellular compartments communicate with each other through a vast and apparently complex traffic of vesicles. The best-studied process is the endocytosis of macromolecules, a property which, like secretion, appears to be constitutive to all cells (see Steinman *et al.*, 1983, for a review). It has been shown that some receptors are interiorized after binding to their respective ligands, dissociated under the effect of the low pH of the "endosomal" compartments, and recycled back to the cell surface. This mechanism implies that a given protein may be found, albeit at different concentrations, in various cellular compartments. The same concept applies to the segregation, during biogenesis, of components destined to the different segments of the secretory pathway (rough and smooth ER, Golgi, plasma membrane). Specific fusion–fission interactions between the different cellular compartments are responsible for conferring selectivity to this movement of material, but no information is available on the molecular details underlying these interactions. Evidence from work with fusogenic viral glycoproteins indicates that restricted amino acid sequences may be responsible for the fusing capacity and that single amino acid substitutions may block it (White *et al.*, 1983). Further work is needed to determine the generality of this mechanism in the fusion between cellular vesicles.

F. Restrictions on Lateral Mobility in the Plane of the Bilayer

Interactions with peripheral or transmembrane proteins which are specific to a particular membrane and which do not participate in recycling may be an important mechanism to stabilize the composition of a given membrane. All proteins belonging to such a membrane would share "retention signals" (specific se-

quences or conformational features) that recognize the primary organellar structural protein which, in order for the system to work, must possess the ability to interact with other similar molecules. Organelles connected by flow of membrane material (such as the different segments of the secretory pathway) would be, according to this view, composed of one or more primary structural proteins (probably conferring the shape of the organelle), associated secondary proteins (carrying out the specific functions of the organelle), and proteins in transit to other organelles. A beautiful example of the interactions described in this paragraph is provided by the work of Branton and colleagues, describing the relationship of peripheral proteins in red blood cells, such as ankyryn and spectrin, with integral membrane proteins and the cytoskeleton (Branton *et al.*, 1981). Another possible example is the ribophorins I and II, which have been postulated to carry the ribosome binding sites and perhaps to be responsible for the typical shape of the rough ER (Kreibich *et al.*, 1978a-c).

Restrictions to the lateral mobility in the plane of the bilayer may also be caused by interaction with extracellular molecules, such as collagen, fibronectin, or laminin, or by the presence of uninterrupted discontinuities in the bilayer, such as the occluding junctions that separate the apical and basolateral regions of the epithelial cell plasmalemma (see Section IV,C,2).

G. Lipid Sorting

Most of the terminal steps in phospholipid and cholesterol synthesis are carried out by enzymes in the ER (van Golde *et al.*, 1974; Jelsema and Morre, 1978; Op de Kamp, 1979; Bell and Coleman, 1980). However, cholesterol is almost absent from ER membranes (cholesterol:phospholipid ratio, 1:10), has intermediate concentrations in the Golgi apparatus, and is a major component of plasma membranes (cholesterol:phospholipid ratio, 1:1). The factors that govern these differential lipid distributions in specific subcellular locations are completely unknown. Equally unknown are the mechanisms responsible for the asymmetric localization of phospholipids in the plasma membrane: aminophosphatides in the inner half, choline phosphatides and glycolipids in the outer half (Bretscher, 1973; Verkley *et al.*, 1973; Renooij *et al.*, 1974; Rothman and Lenard, 1977). Other important questions are as follows: Do all or only some of the cell membranes have the necessary machinery to generate lipid asymmetry? Are the intracellular pathways for lipids the same or do they differ to a certain extent from those followed by the proteins? What is the biological role of lipid asymmetry? The observation that fluorescent lipid analogs adopt specific surface and intracellular distributions, depending on the lipid, and are transported and metabolized within the cell (Pagano and Longmuir, 1983) opens the possibility of providing answers to some of these questions.

III. MOLECULAR SORTING IN THE SECRETORY PATHWAY

A. The Secretory Pathway

It is now well documented that the secretory pathway not only functions as a protein export system but is also a main molecular distribution system in the cell. Originally described in glandular epithelial cells, it is found, although modified, in every eukaryotic cell type (Palade, 1975), including yeast. Secretory proteins undergo the following biosynthetic and processing steps (Jamieson and Palade, 1971, 1977; Palade, 1975): (1) synthesis of a precursor (or preprotein) by polyosomes attached to the outer surface of the rough ER; (2) segregation into the ER cisternae, mediated by a signal sequence; (3) intracellular transport via transitional smooth ER vesicles to the Golgi complex; (4) concentration and storage in secretory granules in cells with "regulated" secretion; this step is absent in cells with continuous or "nonregulated" secretion; and (5) exocytosis.

B. Sorting in the Endoplasmic Reticulum

There is now a large body of evidence indicating that the secretory pathway participates in the synthesis, processing, and distribution of lysosomal hydrolases and plasma membrane glycoproteins. Although it is believed that the same is true for integral membrane proteins of ER, Golgi, lysosomes, and peroxisomes, no experimental proof is available because of the unavailability of purified markers. The ER appears to possess a population of intrinsic transmembrane glycoproteins (Rodriguez-Boulan *et al.*, 1978a,b), with their carbohydrate residues exposed in the luminal surface (Hirano *et al.*, 1972). Lectins specific for mannose or glucose residues (such as concanavalin A), but not lectins with affinity for branch carbohydrates (such as ricin or wheat germ agglutinin) bind to the luminal side of ER membranes (Hirano *et al.*, 1972; Rodriguez-Boulan *et al.*, 1978a). Only two integral ER glycoproteins have been characterized in some detail: ribophorins I and II, believed to be the ribosome attachment sites (Kreibich *et al.*, 1978a-c). The membrane glycoproteins of viruses that bud from the RER, such as the rotaviruses (see Section IV,C,3), or of mutants of surface-budding viruses with defects in the exit of the glycoproteins from the ER (Lafay, 1974; Knipe *et al.*, 1977a; Lohmeyer and Klenk, 1979; Rodriguez-Boulan *et al.*, 1984), should provide a useful system to study the biogenesis of ER integral transmembrane proteins.

From a biogenetic point of view, it is important to elucidate what factors determine that some proteins remain in the ER after synthesis, while others move ahead to the Golgi, lysosomes, or plasma membrane. Is it the possession of a

“retention signal” (see Section II,F) in ER proteins designed to interact with other ER-specific proteins or is it the lack of “addressing signals” designed to interact with specific mechanisms that mediate the transport away from the ER? Different secretory or membrane proteins are transported from ER to the Golgi at very different rates, a finding which has been interpreted as meaning that one or more specific receptors, presumably carbohydrates, are involved in this process (Strous and Lodish, 1980; Lodish *et al.*, 1983; Lodish and Kong, 1984; Fitting and Kabat, 1982; Ledford and Davis, 1983). An alternative explanation of these experiments would be that secretory proteins differ in the strength of their interactions with ER components, a characteristic which results in different retention times in this organelle. It is known that some ER proteins can be detected, albeit at reduced concentrations, in the Golgi apparatus (Ehrenreich *et al.*, 1973; Ito and Palade, 1978), possibly in the proximal or *cis* cisternae. It is possible that multiple mechanisms coexist and determine transport between ER and Golgi: Retention signals in ER proteins may prevent their diffusion away from the ER; those that escape and are carried to the Golgi together with proteins destined to other organelles are recycled back to the ER because of the lack of “addressing signals” that determine the compartmentalization into specific post-Golgi vesicles. A discussion of some recent molecular data on the movement of proteins between the ER and Golgi, provided by recombinant DNA work, can be found in Section IV,E.

C. Sorting in the Golgi Apparatus

The role of the Golgi apparatus as a major sorting center in the cell is well recognized (Farquhar and Palade, 1981; Rothman, 1981; Farquhar, 1983). The Golgi apparatus is ideally placed at the crossroads of the exocytic and endocytic pathways to carry out this function. The structure and function of this organelle have been extensively reviewed (Whaley and Dauwalder, 1979; Tartakoff, 1980; Farquhar and Palade, 1981). It consists of a stack of 4–10 smooth-surfaced cisternae, usually with dilated rims, associated vesicles (some of them coated), and vacuoles. Morphologically, the Golgi complex sometimes displays a clear polarity, with the proximal or *cis* cisternae facing the endoplasmic reticulum and the distal or *trans* cisternae facing either the nucleus or the plasmalemma (because of a general spiral or coiled organization). Membrane thickness increases progressively from proximal to distal cisternae. Biochemical polarity is evident in the differential enzyme distribution, detected by cytochemical and immunocytological procedures [thiamine pyrophosphatase (TPPase), nucleoside diphosphatase (NDPase), and galactosyltransferase are markers of the distal cisternae (Novikoff *et al.*, 1971; Novikoff and Novikoff, 1977; Roth and Berger, 1982)], and in the asymmetric distribution of lectin-binding sites [*Ricinus*

communis agglutinin, with affinity for galactose residues, binds preferentially to the distal cisternae in some cell types (Griffiths *et al.*, 1982; Tartakoff and Vassali, 1983)].

Major enzymatic activities associated with the Golgi stack are glycosidases involved in the trimming of carbohydrate residues from core oligosaccharides, transferases for addition of *N*-acetylglucosamine, galactose, fucose, and sialic acid to glycoproteins (Parodi and Leloir, 1979; Schachter and Roseman, 1980; Lennarz, 1980), transferases for the incorporation of galactose and sialic acid to glycolipids (cerebrosides and gangliosides), sulfotransferases, and proteases involved in the processing of proproteins (Young, 1973; Steiner *et al.*, 1970; reviewed in Farquhar and Palade, 1981). The two enzymes responsible for the addition of mannose 6-phosphate groups to lysosomal hydrolases, an *N*-acetylglucosaminyltransferase and a phosphodiesterase, also appear to be localized in the Golgi complex (Varki and Kornfeld, 1980; Reitman and Kornfeld, 1981). More recently, four initial Golgi enzymes of the glycosylation pathway (mannosidase I, *N*-acetylglucosaminetransferase I, mannosidase II, and *N*-acetylglucosaminetransferase II) were resolved from two late enzymes (galactosyltransferase and sialyltransferase) by sucrose-gradient centrifugation (Dunphy and Rothman, 1983).

These typical enzymatic activities of the Golgi apparatus have been very useful in assessing the passage of various classes of proteins through the organelle. As a consequence of the addition of terminal carbohydrates, glycoproteins become resistant to the action of endoglycosidase H; this provides a simple test for the passage of some glycoproteins through the Golgi complex (Robbins *et al.*, 1977). It is now clear that secretory proteins, plasma membrane glycoproteins, and lysosomal hydrolases undergo biochemical modifications typical of passage through the Golgi apparatus (Farquhar and Palade, 1981; Rothman, 1981; Strawser and Touster, 1980; Sly, 1982). Coated vesicles have been postulated to participate in the post-Golgi transport of plasma membrane proteins (Rothman and Fine, 1980; Rothman *et al.*, 1980) and lysosomal hydrolases (Friend and Farquhar, 1967). A special cellular structure associated with the ER and the Golgi, or GERL, has been proposed on the basis of cytochemical evidence as an organelle involved in the biogenesis of lysosomal hydrolases (Novikoff, 1976).

Although no structural or biogenetic information is available on intrinsic Golgi proteins, it may be assumed that transmembrane glycoproteins in the organelle are inserted into the ER membrane via signal sequences and then transported to the Golgi apparatus, as shown for plasma membrane glycoproteins (see Section III,D and E). At least three Golgi proteins, including the classic marker galactosyltransferase, have already been purified (Louvard *et al.*, 1982; Roth and Berger, 1982) and antibodies against them have been raised and used for immunolocalization studies. It has been shown, using antibodies against galac-

tosyltransferase, that this typical Golgi enzyme is synthesized in the ER and transported to the Golgi apparatus in approximately 20 minutes, where it stays for an average of about 20 hours (Strous *et al.*, 1983; Strous and Berger, 1982).

D. Sorting of Lysosomal Proteins

Lysosomal hydrolases are synthesized as larger precursors and inserted via signal sequences into the ER lumen (Erickson and Blobel, 1979; Hasilik and Neufeld, 1980a; Rosenfeld *et al.*, 1982). Like secretory proteins, they are cotranslationally glycosylated by transfer of a GlcNAc₂-Man₉-Glc₃ oligosaccharide from dolichol phosphate. The glucose residues are trimmed by specific glycosidases in the ER, and *N*-acetylglucosamine 1-phosphate is added to the 6 position of several mannose residues of the high-mannose oligosaccharides (Tabas and Kornfeld, 1980; Hasilik and Neufeld, 1980b). Subsequently, a phosphodiesterase removes, probably in the Golgi, the *N*-acetylglucosamine residues and exposes the phosphate groups on the mannose residues (Varki and Kornfeld, 1980; Hasilik *et al.*, 1981). These mannose 6-phosphate groups are recognized by specific receptors, presumably in the Golgi apparatus or GERL region, that mediate the transfer to the lysosomes (see Sly, 1982, for a recent review). Recently, Brown and Farquhar (1984) have localized these receptors with monospecific antibodies and immunoelectron microscopy to cisternae and coated vesicles in the cis Golgi apparatus. Mannose 6-P groups also mediate an alternative pathway of the hydrolases to the lysosomes: the uptake from the medium via receptor-mediated endocytosis. The relationship between the receptors that mediate this secretion-recapture pathway (Neufeld *et al.*, 1977) and the intracellular receptors for mannose 6-P is not yet totally understood.

E. Sorting of Plasma Membrane Glycoproteins

The last decade has seen an explosive increase in information on the structure and biogenesis of plasma membrane proteins. The availability of DNA cloning and sequencing techniques is accelerating this process even further. Table II summarizes structural and biogenetic data for some of the best-studied plasma membrane glycoproteins.

It is now well established that, like secretory proteins, plasma membrane glycoproteins are (1) produced by polysomes bound to the ER and inserted cotranslationally into its membrane via signal sequences (Rothman and Lodish, 1977; Garoff *et al.*, 1978; Lingappa *et al.*, 1978; Dobberstein *et al.*, 1979; Krangel *et al.*, 1979); (2) cotranslationally glycosylated by transfer in block from dolichol phosphate of a core oligosaccharide, GlcNAc₂-Man₉-Glc₃, which is later trimmed and further processed by ER and Golgi enzymes to the simple or

complex residues found in mature glycoproteins (Hunt *et al.*, 1978; Leblond and Bennet, 1977; Lennarz, 1980; Parodi and Leloir, 1979; Robbins *et al.*, 1977; Schachter and Roseman, 1980; Tabas *et al.*, 1978; Hubbard and Ivatt, 1981); (3) subject to additional processing, such as proteolytic cleavage, sulfation, and covalent linking of lipids (Lazarowitz and Choppin, 1975; Nakamura and Compans, 1977; Schmidt and Schlessinger, 1979). A major difference from secretory proteins is the possession of additional hydrophobic sequences responsible for the anchoring to the lipid bilayer (Garoff and Soderlund, 1978; Kehry *et al.*, 1980; Rose *et al.*, 1980; Gething and Sambrook, 1982; Sveda *et al.*, 1982).

Because of the large amounts produced by the infected cells and other experimental advantages (see Section IV.C.1), the membrane glycoproteins of three enveloped viruses—vesicular stomatitis (VSV) G protein, Semliki Forest virus E1, E2, and E3 envelope proteins, and the influenza hemagglutinin (HA)—are the best-characterized members of the group (Lenard, 1978; Simons and Garoff, 1980). Morphological details of the intracellular pathway are available, in fact, only for them (Bergmann *et al.*, 1981; J. Green *et al.*, 1981; Wehland *et al.*, 1982; Bergeron *et al.*, 1982; Rindler *et al.*, 1982; Rodriguez-Boulan *et al.*, 1984). These studies have confirmed previous biochemical evidence: the glycoproteins are detected initially in the ER, 10 minutes later in the Golgi apparatus, and 30–40 minutes later in the cell surface. Although these major steps are clear, some confusion remains regarding the nature of the intermediate carrier vesicles. Evidence obtained by cell fractionation experiments suggests the participation of coated vesicles in the transport of G protein between ER and Golgi, and between Golgi and plasma membrane (Rothman and Fine, 1980; Rothman *et al.*, 1980). A morphological demonstration, however, was not available. In fact, in immunoelectron microscopy experiments using a temperature-sensitive mutant of influenza, with a reversible block in the HA exit from the ER that was used to synchronize its migration, the vesicles that became labeled at intermediate times between Golgi and the cell surface were smooth and larger than coated vesicles (Rodriguez-Boulan *et al.*, 1984).

In spite of the considerable amount of information on the structure and processing of plasma membrane glycoproteins, very little is known about how their sorting is carried out. Different glycoproteins exhibit different orientations in the plasma membrane; the most frequent is amino-terminal end in the external domain, carboxy-terminal end in the cytoplasmic domain (Table II). Orientation, though, is not even related to finer degrees of sorting since, in epithelial cells, two proteins with the same orientation (HA and VSV G protein) are distributed to different surfaces (apical and basolateral, respectively), whereas proteins with opposite orientation (HA and neuraminidase of influenza virus, and sucrose-isomaltase) go to the same surface (apical) (Rodriguez-Boulan and Sabatini, 1978; Rodriguez-Boulan, 1983; Srinivas *et al.*, 1983; see Table II). In contrast to their role in lysosomal proteins, carbohydrates do not seem to be part of the

TABLE II. BIOGENETIC DATA

Glycoprotein	Molecular weight (kDa)	N	Orientation		Signal sequence		Length (amino acids)
			N-ter	C-ter	Localization	Cleav.	
Viral glycoproteins							
VSV G	61	3	Ext	Cyt	N-ter	Yes	16
Influenza							
HA	74	3	Ext	Cyt	N-ter	Yes	16-18
NA	~50	4	Cyt	Ext	N-ter	No	~30
SVF							
E1	51	3	Ext	Cyt	N-ter	Yes	14
p62	62	3	Ext	Cyt	N-ter	No	12
Histocompatibility antigens							
Mouse H-2	44	2	Ext	Cyt	N-ter	Yes	15-20
Human HLA (heavy chain)	41	2	Ext	Cyt	N-ter	Yes	20-24
Immunoglobulins							
Membrane IgM, IgG (heavy chain)	70	2	Ext	Cyt	N-ter	Yes	18
Erythrocyte glycoproteins							
Glycophorin	30	?	Ext	Cyt	N-ter	Yes	?
Band 3	93	2	Cyt	Ext	Internal	No	?
Plasma membrane proteins of polarized cells							
Sucrase-isomaltase	150	2	Cyt	Ext	N-ter	No	~20
Aminopeptidase	130	2	Cyt	Ext	N-ter	No?	33-42
Receptors							
IgA	82	?	Ext	Cyt	N-ter	Yes	18
Acetylcholine	250	5	Ext	Cyt(?)	N-ter	Yes	17-24

^a Key to references: (1) Mudd (1974); Lingappa *et al.* (1978); Robbins *et al.* (1977); Tabas *et al.* (1978); Hunt *et al.* (1978); Rose *et al.* (1980); Lodish *et al.* (1981); (2) Ward and Dopheide (1979); Waterfield *et al.* (1979); Porter *et al.* (1979); Hiti *et al.* (1981); Gething *et al.* (1980); Sleigh *et al.* (1980); Min Jou *et al.* (1980); Davies *et al.* (1980); Wilson *et al.* (1981); Winter *et al.* (1981); (3) Colman *et al.* (1983); Fields *et al.* (1981); Blok *et al.* (1982); Varghese *et al.* (1983); (4) Garoff and Soderlund (1978); Garoff *et al.* (1980); Simons and Garoff (1980); (5) Dobberstein *et al.* (1979);

sorting signals of plasma membrane glycoproteins, since they reach the cell surface—even the correct surface domain (apical or basolateral) in epithelial cells—in the presence of tunicamycin, an inhibitor of ASN-linked glycosylation (Gibson *et al.*, 1978; Roth *et al.*, 1979; Strous and Lodish, 1980; R. F. Green *et al.*, 1981). Ionophores, such as monensin, have been shown to inhibit the transport to the cell surface (Tartakoff and Vasalli, 1977; Johnson and Schlesinger,

ON PLASMA MEMBRANE GLYCOPROTEINS

n	Anchor pieces		Carbohydrates			Migration half-time (min)	Surface localization in epithelia	Ref. ^a
	Localization	Length (amino acids)	Percentage	Type	Proteolytic fragments			
1	C-ter	20	10	S,C	No	50	Basal	1
1	C-ter	24-30	19	S,C	HA1, HA2	60	Apical	2
1	N-ter	~30	20	S,C	No	>2 hours	Apical	3
1	C-ter	27	7	S,C	No	50	Basal	4
1	C-ter	32	9	S,C	E2, E3	50	Basal	4
1	C-ter	24	8	C	No	60	Basal	5
1	C-ter	10	4	C	No	60	Basal?	6
1	C-ter	26	15	S,C	No		?	7
1	C-ter	12	60	O,C	No	40	?	8
3	—	13	10	C	No		?	9
1	N-ter	~20	15	S,C	S,1	45	Apical	10
1	N-ter	33-42	13-35	S,C	No	—	Apical	11
1	C-ter	23	—	—	15,69 kd	30-60	b-Apical	12
~4	—	18-30	3	—	No	~3 hours	?	13

Pease *et al.* (1982); Ploegh *et al.* (1981); Evans *et al.* (1982); (6) Parham *et al.* (1977); Ploegh *et al.* (1979, 1981); Krangel *et al.* (1979); Owen *et al.* (1981); (7) Rogers *et al.* (1980); (8) Tomite and Marchesi (1975); Furthmayr (1977); Jokinen *et al.* (1979); (9) Steck (1978); Braell and Lodish (1982); (10) Semenza (1976); Brunner *et al.* (1979); Frank *et al.* (1978); Hauri *et al.* (1979, 1982); (11) Lojda (1972); Kenny and Maroux (1982); (12) Kraehenbuhl and Kuhn (1978); Solari and Kraehenbuhl (1984); Mostov *et al.* (1984); (13) Noda *et al.* (1982, 1983a,b); Claudio *et al.* (1983).

1980; Kaariainen *et al.*, 1980; Tartakoff, 1983), presumably by interfering with translocation within the Golgi (Griffiths *et al.*, 1983). Low temperature (20°C) blocks a later transport step, presumably at the level of post-Golgi vesicles (Matlin and Simons, 1983). Mutants of enveloped viruses in which membrane glycoproteins fail to reach the cell surface do not fit into a consistent pattern so far. Mutations of a vaccinia virus-coded surface glycoprotein which result in

extra amino acids in the cytoplasmic domain are retained in the ER (Shida and Matsumoto, 1983) but deletion of the cytoplasmic domain of G protein (Rose and Bergman, 1982), HA (Gething and Sambrook, 1981; Sveda *et al.*, 1981), or Semliki Forest virus glycoproteins (Garoff *et al.*, 1983) by recombinant DNA technology does not affect their expression via the appropriate vectors (see Section IV,D). On the other hand, mutant cell lines which fail to produce β_2 -microglobulin do not express HLA on the cell surface (Ploegh *et al.*, 1979). Since β_2 -microglobulin interacts with the ectoplasmic domain of HLA in the ER, this observation suggests a role for this domain in the sorting of HLA. Obviously, interpretation of any data as discussed above must take into account possible changes in conformation in the protein tertiary or even quaternary structure (since all integral membrane proteins seem to be in the state of, at least, dimers; see Table II). Recent data on recombinant DNA work related to the sorting of viral envelope glycoproteins are discussed in Section IV,D.

F. Molecular Sorting, Endocytosis, and Membrane Recycling

Whereas some proteins (such as secretory proteins) undergo only one round of sorting during their biogenesis, it is clear that other proteins are subject to many rounds of recycling through various cellular compartments (and therefore, to many sorting events) during their lifetime. The best-known example are some plasma membrane glycoproteins, in particular various receptors to hormones, growth factors, and viruses, which are removed from the cell surface to be quickly incorporated into intracellular vesicles, called "endosomes" or "receptosomes" (Helenius *et al.*, 1983; Pastan and Willingham, 1983), and recycled back to the plasmalemma. The physiological role of recycling through intracellular compartments, in addition to allowing the endocytosis of various types of molecules or particles, appears to be in many cases the dissociation of ligand from receptor (e.g., low-density lipoprotein, epidermal growth factor, asialoglycoprotein, and α_2 -macroglobulin receptors) or of an essential factor from its carrier protein (such as iron from transferrin), which allows the reutilization of the receptor or carrier protein for subsequent rounds of endocytosis or transport (Anderson and Kaplan, 1983). Dissociation is triggered by the existence of a low pH (5–6) in the endosomal compartment (Tycko and Maxfield, 1982), possibly generated by a special proton pump present in endosomes, coated vesicles, lysosomes, and even in the Golgi apparatus (Schneider, 1981; Forgac *et al.*, 1983; Marsh *et al.*, 1983; Glickman *et al.*, 1983).

In addition to this "receptor-mediated endocytosis" (Goldstein *et al.*, 1979), cells are able to interiorize large amounts of fluid and dissolved solutes by fluid-phase pinocytosis. The magnitude of the movement of membrane material during

these endocytic processes is very large: fibroblasts interiorize 0.9% and macrophages 3.1% of their cell surface every minute (Steinman *et al.*, 1976). Most of the membrane returns to the cell surface, while the endocytosed fluid and solutes are delivered to the lysosomes, indicating the existence of sorting mechanisms operating at this level. Very little information is available, however, on how specific the movement of membrane material is (i.e., whether only some plasma membrane proteins are subject to recycling) and on what the forces responsible for this process are. Bretscher (1981) has shown that certain major cell surface proteins, such as theta and H63 antigens, appear not to be endocytosed and recycled.

Most of the information available on the routes of membrane internalization come from studies on receptor-mediated endocytosis. Upon ligand binding, receptors cluster in coated pits (or may be preclustered there) (Goldstein *et al.*, 1979; Anderson and Kaplan, 1983; see article by Wiley, this volume), are internalized in coated vesicles, and appear within seconds in uncoated vesicles (endocytic vesicles, "receptosomes," or "endosomes") (Wall *et al.*, 1980; Helenius *et al.*, 1983; Pastan and Willingham, 1983; Steinman *et al.*, 1983). These vesicles display saltatory movement in the cytoplasm and fuse in 2–20 minutes with lysosomes (Pastan and Willingham, 1983) in a process inhibited by incubation at temperatures below 20°C (Dunn *et al.*, 1980). The routes of membrane recycling vary according to the cell type. While many ligands and fluid-phase proteins are delivered to the lysosomes, passage through the Golgi apparatus has been described (Herzog and Farquhar, 1977; Herzog and Miller, 1979; Farquhar, 1982; Pastan and Willingham, 1983). In epithelial and endothelial cells, mechanisms exist to transfer proteins from one to the other side of the monolayer, bypassing the lysosomes. Examples of this process are the transfer of maternal IgG via Fc receptors from the luminal to the basal medium in newborn rat intestine and the transport of IgA via a membrane receptor that is cleaved and released as a complex with the immunoglobulin (secretory component) in various glandular epithelia (Rodewald, 1973, 1980; Kraehenbuhl and Kuhn, 1978; Mostov *et al.*, 1984).

From the viewpoint of this article, it is important to stress that many sorting steps take place during this movement of membrane material: sorting of plasma membrane proteins that enter into coated vesicles and endosomes from those that stay in the cell surface, sorting of receptor from ligand in the endosomes, sorting of endosomal membrane proteins from receptors that return to the cell surface, sorting of endosomal fluid phase proteins to the lysosomes, sorting of proteins transported across the monolayer from those delivered to the lysosomes in epithelial cells. Very little is known about the nature of the signals that mediate these sorting events. The best-known sorting signal for endocytosed ligands is also one of the best-characterized signals of exocytic processes: the mannose 6-P group of lysosomal hydrolases (Sly, 1982). No common feature of recycling

receptors has been found so far; carbohydrates do not appear to be involved (Brown *et al.*, 1983). Regarding the nature of the structures that recognize the sorting information in the proteins, a large amount of circumstantial evidence has established an important role for clathrin-coated vesicles (Anderson and Kaplan, 1983; Brown *et al.*, 1983). Geuze *et al.* (1983) have provided immunoelectron microscopic evidence indicating that, after dissociation from the ligand in the endosomes, receptors are segregated into a population of thin tubular vesicles designated by the acronym CURL (compartment of uncoupling of receptor and ligand). This mechanism provides a very simple possible explanation of how the bulk of the endosomal volume containing dissociated ligand destined to the lysosomes is segregated from the receptors, which return in small-volume vesicles to the cell surface.

Another important unsolved question is the extent to which the exocytic and endocytic pathways overlap. Do plasma membrane proteins being delivered from Golgi to the cell surface for the first time travel through the endosomal compartment? Recent data from our laboratory (Salas, unpublished results) indicate that anti-influenza hemagglutinin (HA) antibody incorporated into endosomal vesicles of MDCK cells fails to react with newly synthesized HA migrating to the cell surface, suggesting that the two pathways do not overlap.

IV. MODEL SYSTEMS FOR THE STUDY OF MOLECULAR SORTING IN EUKARYOTIC CELLS

A. Reconstitution Systems

A main advantage of reconstitution systems is that they permit direct studies of particular sorting steps. Improvements in cell fractionation, protein separation, and immunoprecipitation procedures in the last decade have made them possible. They are currently used to study the translocation of proteins into the ER or into the nonsecretory organelles (mitochondria, chloroplasts, nucleus) and the movement of proteins between Golgi subcompartments. Theoretically, they could be applied to the study of other sorting steps in the secretory pathway; the main limitation is imposed by the availability of pure subcellular fractions and recognizable biochemical modifications of the molecule under study in the target organelle.

1. *In Vitro* TRANSLATION-TRANSLOCATION SYSTEMS

The development of an *in vitro* translation system that translocated immunoglobulin light chains across dog pancreas microsomal membranes (Blobel and Dobberstein, 1975a,b) constituted a major step forward in the elucidation of the

mechanisms responsible for protein translocation across the ER membrane. Further refined to allow synchronization of protein synthesis (Rothman and Lodish, 1977), it provided the first direct proof that the amino-terminal hydrophobic sequences of secretory proteins and integral membrane glycoproteins were somehow responsible for the translocation process. Other important findings with this system were the demonstration that core glycosylation and signal sequence cleavage were cotranslational (Rothman and Lodish, 1977), that some translocatable proteins that lacked cleavable signal sequences had internal segments responsible for vectorial discharge (Lingappa *et al.*, 1978; Braell and Lodish, 1982), and that lysosomal hydrolases were synthesized as precursors and segregated into the ER lumen via signal sequences (Erickson and Blobel, 1979). In recent years, a series of elegant experiments have demonstrated the existence of a ribonucleoprotein complex [the signal recognition particle (SRP)] (Walter and Blobel, 1980, 1982) that arrests protein synthesis through an interaction with the ribosome and the signal sequence (Walter *et al.*, 1981; Walter and Blobel, 1981a,b) and mediates translocation through the ER membrane via attachment to an ER membrane protein (the signal recognition receptor or "docking protein") (Meyer and Dobberstein, 1980a,b; Gilmore *et al.*, 1982a,b; Meyer *et al.*, 1982).

The effect of a hydrophobic sequence spliced into secretory or cytosolic proteins was studied by Yost *et al.* (1983), using a cell-free transcription-linked translation system and recombinant DNA technology. They utilized a hybrid gene coding for a chimeric secretory protein composed of 182 amino acids of bacterial pre- β -lactamase toward the N-terminus, and 142 amino acids of chimpanzee α -globin toward the C-terminus. When its mRNA, transcribed *in vitro*, was translated in a wheat germ system in the presence of dog pancreas microsomal membranes, it directed the synthesis of a protein that was translocated into the microsomal lumen and had its signal sequence removed. Introduction of the anchor sequence of the membrane-bound form of IgM μ chain between the lactamase and the globin resulted in the synthesis of a protein that was also correctly translocated and had its signal peptide removed but remained membrane attached, with the lactamase portion facing the lumen and the globin portion exposed to the cytoplasm, a finding showing that the IgM sequence conserved its anchor function in spite of the long cytoplasmic domain. When the IgM anchor sequence was placed, instead, at the N-terminal end of a cytosolic fusion protein containing mainly globin sequences, the resulting protein was not membrane bound, indicating that the extra hydrophobic segment failed to function both as a signal sequence and as an anchor piece.

In vitro translocation systems have also been used to study the posttranslational transfer of proteins into chloroplasts (Dobberstein *et al.*, 1977; Chua and Schmidt, 1979), mitochondria (see Chua and Schmidt, 1979; Poyton, 1983; and article by Reid, this volume, for reviews), and peroxisomes (Goldman and Blobel, 1978).

2. *In Vitro* SYSTEMS TO STUDY VESICULAR TRANSPORT BETWEEN ORGANELLES

Fries and Rothman have introduced a cell-free system to study the *in vitro* transport of a plasma membrane glycoprotein (the envelope glycoprotein G of vesicular stomatitis virus) to the Golgi apparatus (Fries and Rothman, 1980; Rothman and Fries, 1981). Donor membranes were extracts of a mutant Chinese hamster ovary (CHO) cell line infected with VSV. Because of the lack of UDP-*N*-acetylglucosaminyltransferase I, the G protein in these extracts has carbohydrate residues sensitive to the action of endoglycosidase H. Processing to the typical, endo H-resistant form was observed after addition of exogenous Golgi membranes. Although originally designed to study protein transfer from ER to Golgi, more controlled recent experiments suggest that the G protein is indeed being transported between two different sets of Golgi membranes (Fries and Rothman, 1981). The process requires ATP and cytosolic factors.

In a similar line are the experiments by Altstiel and Branton (1983) involving the fusion *in vitro* of highly purified brain coated vesicles with purified kidney lysosomes. Fusion, followed by the activation of the nonfluorescent compound 6-carboxydiacetylfluorescein to the fluorescent 6-carboxyfluorescein by lysosomal hydrolases, was found to require free calcium and the stripping of the vesicular coat.

B. Genetic Systems

1. SECRETION MUTANTS IN YEAST

Schekman and his collaborators have developed a model system involving the use of mutants of *Saccharomyces cerevisiae* with temperature-sensitive defects in secretory steps, with the aim of identifying the full range of cell functions required for intracellular protein transport (see Schekman, 1982, for a review). The yeast cell surface consists of three layers: a cell wall (consisting of mannoproteins and polysaccharides), a periplasm, and a plasmalemma. Except for small-molecular-weight toxins secreted in the medium, most of the secreted enzymes accumulate in the periplasm. These are exocytosed at the same surface point where new material is added to the surface for bud growth (Tkacz and Lampen, 1972; Field and Schekman, 1980); presumably the same exocytic vesicles are used for surface and secreted molecules (Novick and Schekman, 1983). A striking feature of yeast is the low level of secretory organelles detected by electron microscopy, which correlates with a fast intracellular transport and small precursor pools of secreted proteins (Novick and Schekman, 1979; Novick *et al.*, 1981).

Based on the observation that ts secretory mutants are denser than normal cells

when incubated at the nonpermissive temperature (37°C) more than 200 *sec* mutants were isolated; these fell into two major classes: A and B, with 23 and 4 loci, respectively (Novick *et al.*, 1980; Ferro-Novick *et al.*, 1984a,b). In all mutants, secretion and surface growth stopped at 37°C. Group A *sec* mutants continued to synthesize and accumulate active secretory enzymes; interestingly, accumulation resulted in the exaggeration of the specific organelle behind the block (ER, Golgi-like, vesicles), where acid phosphatase (a secretory enzyme) could be detected by immunocytochemistry. Class B *sec* mutants produce enzymatically inactive forms of invertase and acid phosphatase; in two of them (*sec53* and *sec59*) immunoreactive forms of invertase are found embedded in the ER membrane, presumably as a consequence of a defect in function required for the completion of translocation (Ferro-Novick *et al.*, 1984a,b). Studies of double *sec* mutants demonstrated the following sequence of mutant functions: B—A (ER)—A (Golgi bodies)—A (vesicles), in which the mutants at the left are epistatic to the other mutants.

Glycosylation of yeast glycoproteins apparently proceeds in two compartmentalized steps, similar to those observed in mammalian cells: addition of an N-linked GlcNAc₂-Man₉ core in the ER and of an outer chain of up to 150 mannose residues, presumably in the Golgi apparatus (Esmon *et al.*, 1980; Lehle *et al.*, 1979). Transport to the cell surface of several glycoproteins with asparagine-linked carbohydrates is not inhibited by tunicamycin (Novick and Schekman, 1983). Mutants with a defect in the exit from the ER accumulate glycoproteins with only the core oligosaccharide. Transport of carboxypeptidase Y to the yeast vacuole, a lysosome analog, is, unlike its transport in mammalian cells, independent of carbohydrates (Hasilik and Tanner, 1978; Onishi *et al.*, 1979). It is affected in class B and class A Golgi or pre-Golgi mutants but not in mutants that accumulate (post-Golgi) smooth vesicles (Schekman, 1982).

2. LOW-DENSITY LIPOPROTEIN RECEPTOR MUTANTS

Familial hypercholesterolemia is a rather frequent disease in humans and is caused by defects in the function of the low-density lipoprotein (LDL) receptor of fibroblasts and other cell types (Brown and Goldstein, 1979). This receptor system has provided very important insights on the mechanisms of receptor-mediated endocytosis and the role of coated pits in the movement of membrane material. After binding of LDL at 4°C, the receptors appear randomly distributed on the cell surface; a few minutes after warming to 37°C, the receptors cluster in coated pits and are interiorized within coated vesicles. Under the influence of low pH, the LDLs dissociate from the receptors, presumably in the endosomes (Helenius *et al.*, 1983; Anderson and Kaplan, 1983), and are transferred to the lysosomes for degradation. The receptors are recycled back to the cell surface. Two major genetic defects have been described as causes of familial hyper-

cholesterolemia: a receptor with a very decreased binding capacity and a receptor that has a normal binding capacity but is unable to cluster in the coated pits (and, therefore, to be interiorized).

3. GENES OF SECRETORY AND MEMBRANE-BOUND IMMUNOGLOBULINS

Lymphocytes at different stages of development express genes for secretory or membrane-bound immunoglobulins. Attachment to the membrane in the bound IgM forms is carried out by hydrophobic extra segments in the carboxy-terminal ends (Vassalli *et al.*, 1979; Kehry *et al.*, 1980; Singer and Williamson, 1980; see Table II). Both forms are coded by the same genomic arrangement of exons and introns, which generates two different mRNAs upon differential processing of the RNA transcript (Early *et al.*, 1980). Thus, this system provides an interesting natural example of the role of hydrophobic extra segments in membrane attachment. A similar idea has been explored by deleting the transmembrane segment of integral glycoproteins through recombinant DNA technology (see Section IV,D).

4. LYSOSOMAL PROTEIN MISLOCATION MUTANTS

I-cell disease (mucopolipidosis II) and pseudo-Hurler polydystrophy (mucopolipidosis III) are autosomal recessive lysosomal storage disorders characterized by various skeletal and mental abnormalities and eventually a fatal course (McKusik *et al.*, 1972). A main biochemical feature of these diseases is a high level of lysosomal hydrolases in serum. Cells from these patients show decreased intracellular activities of hydrolases. The biochemical defect appears to be a deficiency of UDP-*N*-acetylglucosaminylphosphotransferase, which prevents the generation of the phosphomannosyl recognition marker of lysosomal enzymes (Reitman *et al.*, 1981; Hasilik *et al.*, 1981) needed for the transfer from Golgi apparatus to the lysosomes. As a consequence, the enzymes are secreted. Lack of the marker prevents the uptake of lysosomal enzymes from the medium (Hickman and Neufeld, 1972; McKusik *et al.*, 1972). Secretion of lysosomal enzymes can also be observed if the synthesis of carbohydrates is inhibited by tunicamycin (von Figura *et al.*, 1979).

C. Other Systems

1. THE GLYCOPROTEINS OF ENVELOPED RNA VIRUSES

A group of plasma membrane glycoproteins, the envelope proteins of enveloped RNA viruses, have proved to be excellent model systems for studies on the biogenesis of plasma membrane glycoproteins (Lenard and Compans, 1974;

Katz *et al.*, 1977; Lenard, 1978; Simons and Garoff, 1980; Lodish *et al.*, 1981). Several experimental advantages have contributed to their popularity: (1) They are produced in large amounts by the infected cells (which allows their easy detection in the cytoplasmic precursor pools). (2) They are processed by cell-coded mechanisms (since the small virus genomes code only for essential viral replicative functions). (3) cDNA complementary to their genomes is relatively easy to obtain and to clone. Therefore, the complete primary structure is known for many viral glycoproteins. Viral envelope glycoproteins have provided an invaluable tool to elucidate the steps and mechanisms involved in the processing of ASN-linked carbohydrates of glycoproteins (see Parodi and Leloir, 1979; Lennarz, 1980, for reviews). Improvements in immunoprecipitation and cell fractionation procedures have allowed the extension of these studies to cellular plasma membrane glycoproteins.

2. POLARIZED BUDDING OF VIRUSES FROM EPITHELIAL CELLS

Epithelial cells infected with enveloped RNA viruses provide an additional advantage for the study of sorting of plasma membrane proteins, since viral glycoproteins may be addressed to one of two possible surface destinations. Enveloped viruses bud with striking polarity from the plasmalemma of polarized epithelial cells: influenza virus, Sendai virus, and Simian virus 5 are assembled from the apical surface of Madin–Darby canine kidney (MDCK) cells, a polarized epithelial line (Misfeldt *et al.*, 1976; Cerejido *et al.*, 1978), whereas VSV is selectively produced from the basolateral surface (Rodriguez-Boulan and Sabatini, 1978; Rodriguez-Boulan, 1983). Polarized budding is preceded, and apparently determined, by the asymmetric insertion of viral glycoproteins into the surface later used for budding (Rodriguez-Boulan and Pendergast, 1980). Influenza hemagglutinin expressed in epithelial cells from a cloned cDNA fragment via SV40 vectors is also asymmetrically distributed (Roth *et al.*, 1983), a finding indicating that nucleocapsid and matrix viral proteins are not necessary for its polarity. Thus, viral glycoproteins share with cellular plasma membrane proteins the sorting signals and biogenetic mechanisms that determine their segregation into different surface domains of epithelial cells.

Neither tunicamycin nor the use of lectin-resistant mutants of MDCK cells or of mutants of influenza virus defective in the neuraminidase alters the polarity of virus budding (Roth *et al.*, 1979; R. F. Green *et al.*, 1981; Meiss *et al.*, 1982). Thus, unlike the mechanism for lysosomal hydrolases, carbohydrates are not part of the sorting signals for viral glycoprotein surface segregation in epithelial cells.

The intracellular pathways of HA and VSV G protein (respectively an apical and a basolateral glycoprotein) are the same until, at least, the Golgi apparatus, as determined by double-labeling immunoelectron microscopy experiments on cells double-infected with influenza and VSV (Rindler *et al.*, 1982, 1984). After

passage through the Golgi, HA is detected in a population of small vesicles that are about twice the size of coated vesicles and that occupy the apical half of the cytoplasm (Rodriguez-Boulán *et al.*, 1984). G protein appears to be predominantly localized into a different population of vesicles in the lower half of the cytoplasm of MDCK cells (Salas and Rodriguez-Boulán, unpublished results). Monensin inhibits the production of the basolateral virus VSV but only delays the production of the apical virus influenza (Alonso and Compans, 1981; Rodriguez-Boulán *et al.*, 1984). Treatment with cytochalasin D or colchicine does not alter the polarized viral budding, a finding indicating that microtubules or microfilaments (at least those sensitive to the drugs) are not involved in polarized viral budding (Salas *et al.*, 1985). Furthermore, addition of anti-HA antibody only to the basolateral surface of confluent MDCK monolayers do not affect the production of influenza virus or the delivery of HA to the apical surface (Misek *et al.*, 1984). These experiments suggest that apical and basolateral glycoproteins are segregated at the level of the Golgi apparatus, by the incorporation into different sets of vesicles that fuse directly with the respective target domains in the cell surface.

There is experimental evidence, however, indicating that some sorting of epithelial plasma membrane proteins may occur at the cell surface. Experiments by Matlin *et al.* (1983) and Pesonen and Simons (1983) show that, after fusion of VSV to the apical surface of MDCK cells, the G protein is interiorized and quickly redistributed to the basolateral domain. Rodewald (1973) has previously shown that IgG is transported from the apical to the basolateral domain of newborn rat intestinal cells by receptor-mediated endocytosis; the fluid marker horseradish peroxidase, added simultaneously to the apical medium, is transported, however, to the lysosomes (Abrahamson and Rodewald, 1981). Transport of IgA in the opposite direction via a specific receptor by several epithelia is well documented (see Kraehenbuhl and Kuhn, 1978, for a review). This receptor is used only once; available evidence suggests that it is delivered first to the basolateral membrane, where it binds IgA, and then migrates to the apical surface, where it is cleaved by specific proteases and released to the medium complexed with IgA (Solari and Kraehenbuhl, 1984). Mostov *et al.* (1984) have cloned DNA complementary to IgA receptor mRNA and determined its complete primary structure (its main features are summarized in Table II); an interesting observation derived from this study is the finding of amino acid sequences homologous to immunoglobulin chains. Hauri *et al.* (1979) have proposed, on the basis of pulse-chase analysis of subcellular fractions, that sucrase-isomaltase, an apical glycoprotein in intestinal epithelium, is first inserted in the basolateral membrane, retrieved, and then transported to the apical surface. However, since the contamination of basolateral membrane fractions with Golgi membranes, or other post-Golgi vesicles, cannot be discarded, these results must be interpreted with caution. The preceding data taken together strongly suggest

the existence of mechanisms to relocate molecules from one surface to the other in epithelial cells, mechanisms which may contribute to surface polarity.

The role of tight junctions and substrate attachment on epithelial polarity has been investigated. Isolated epithelial cells in suspension culture lose, to a large extent, their surface polarity (Pisam and Ripoché, 1976; Ziomek *et al.*, 1980; Rodriguez-Boulan *et al.*, 1983). Attachment to a substrate, however, results in the recovery of the ability to sustain polarized viral budding by MDCK cells, even in the absence of complete tight junctions (Rodriguez-Boulan *et al.*, 1983). The polarity of thyroid cells in monolayer culture can be changed by polymerization of a collagen gel on top of the cells (Chambard *et al.*, 1981). Thus, the interaction with substrate material, which most probably causes a restriction in the mobility of specific surface receptors, reorganizes the epithelial cell surface and contributes to the sorting of the plasma membrane glycoproteins. Complete surface segregation, however, may require the existence of functional tight junctions.

3. ENVELOPED VIRUSES THAT BUD FROM INTERNAL MEMBRANES

Two groups of RNA viruses bud from internal membranes: coronaviruses and rotaviruses (Holmes, 1983; Sturman and Holmes, 1983).

Coronaviruses bud into intracytoplasmic vesicles from the RER and Golgi. One member of the group, mouse hepatitis virus (MHV) possess two glycoproteins: E1, which is the matrix protein and is a transmembrane glycoprotein with its N-terminus facing the outside of the virion and the C-terminus associated with the nucleocapsid, and E2, which is the spike protein and forms the peplomers on the virion surface, incorporated late in the assembly. Tunicamycin blocks the glycosylation of E2, which has a N-linked carbohydrate moiety, but not of E1, which has only O-linked oligosaccharides. Glycosylation of E2 occurs during its cotranslational translocation, via a signal sequence, across the ER membrane. E1 does not present a cleavable signal sequence and apparently is directed toward the membrane by an internal hydrophobic sequence. Glycosylation of E1 occurs late, in the Golgi, and is blocked by monensin, which produces accumulation of enveloped virions in RER vesicles. It seems that the site of budding is determined by inability of E1 to exit from the RER. Similar results were obtained with bovine and human coronaviruses, but avian infectious bronchitis virus presents N-linked oligosaccharides in both glycoproteins.

Knowledge about rotavirus assembly is far from complete. Different laboratories do not agree on how many of the proteins that have a synthesis directed by the viral genome are structural. Mature virions present a double protein capsid; the outer capsid can be removed by calcium-chelating agents (EDTA or EGTA), thereby rendering the virions unable to infect cells. There is no general agreement among investigators about the detailed composition of the capsids; it is

accepted that the inner capsid contains at least one major and two minor proteins (p116, p96, and p42) and that the major component of the outer capsid is a glycoprotein (gp34), together with a nonglycosylated protein (p84) which is cleaved after the virions are released from the cell into peptides (p62 and p28). Several groups would add two or three proteins to the inner capsid and one or two to the outer capsid. In infected cells the synthesis of viral components takes place in viral inclusions, defined by electron microscopy, called "viroplasm" which are similar to the "virus factories" in reovirus-infected cells. Single-shelled virions (i.e., virions containing only the inner capsid) bud from the RER membranes into the lumen and possess a lipid bilayer envelope ("pseudoenvelope") that has to be eliminated to allow the formation of the outer capsid. The viral genome codes for two glycoproteins: gp34, a component of the outer capsid, and gp25, apparently a nonstructural component. Both glycoproteins are found inserted in the RER membrane, and both possess endoglycosidase H-sensitive carbohydrate moieties. The elimination of the pseudoenvelope needs the synthesis of a glycosylated component; in infected cells treated with tunicamycin, the pseudoenvelopes remain around all the particles in the RER vesicles; and upon lysis of the cells, virions with only the inner capsid are released. It has been postulated that the calcium concentration within the RER lumen is sufficient to allow the assembly of the outer capsid upon removal of the pseudoenvelope, but still, the mechanism of translocation across the RER membrane of the non-glycosylated protein(s) of the outer capsid remains unexplored.

4. SORTING INTO CONSTITUTIVE OR REGULATED PATHWAYS

Kelly and his colleagues have presented evidence that AtT-20, a mouse pituitary tumor line that secretes adrenocorticotropin (ACTH) and β -endorphin through a regulated pathway, externalizes a plasma membrane glycoprotein and a high-molecular-weight precursor of both hormones via a different constitutive pathway (Gumbiner and Kelly, 1982; Moore *et al.*, 1983a). Different vesicles appear to be mediating both types of secretion. Chloroquine, which raises the pH of intracellular vesicles, blocks the storage of newly synthesized ACTH into secretory granules and instead diverts it to the outside of the cell via the constitutive pathway (Moore *et al.*, 1983b). This is similar to previous observations with lysosomal hydrolases, which are secreted in the presence of chloroquine (Gonzalez-Noriega *et al.*, 1980).

5. RECEPTOR SORTING DURING ENDOCYTOSIS

This subject has been extensively reviewed (see, for example, Brown *et al.*, 1983; Anderson and Kaplan, 1983; and articles by Wiley and Schneider *et al.*, this volume).

D. Recombinant DNA Technology Applied to the Study of Protein Sorting

The relatively new technologies of molecular cloning and genetic engineering, in combination with the availability of efficient vectors for the expression of cloned genes in eukaryotic cells, have introduced the possibility of studying (and eventually modifying) at the molecular level the nature of protein-sorting signals.

Copy DNA genes need to be ligated to a promoter recognized by eukaryotic polymerase in order to be expressed in eukaryotic cells. A variety of expression vectors carrying viral promoters and other regulatory sequences have been used successfully (see Gluzman, 1982; Rigby, 1982). They were derived from different eukaryotic viruses: SV40, adeno-, papilloma-, and retroviruses. They fall into two main classes: plasmid-type and virus-type vectors. Plasmid vectors are introduced by transfection, a process usually resulting in transient expression of the gene by a small percentage ($< 10\%$) of the cells. On the other hand, infection with viral vectors results in almost all cells expressing the gene at high levels. The most popular viral vector has been SV40 for obvious reasons: the circular genome is relatively small and its complete sequence is known, the promoters and splicing sites have been accurately mapped, all the structural genes have been identified, and it is easy to purify (Elder *et al.*, 1981).

The first report of successful expression of a viral membrane protein using a recombinant vector was published in 1981 by Moriarty *et al.* (1981). A genomic fragment of hepatitis B virus containing the coding sequence for the viral surface antigen (HBA) was incorporated into a defective SV40 genome and used to transfect African green monkey kidney (AGMK) cells previously infected at the nonpermissive temperature with an SV40 early *ts* mutant in order to obtain a stock recombinant virus. Infection of AGMK cells with this stock virus resulted in the production of HBA by 45% of the cells as judged by immunofluorescence. HBA was released into the medium in 22-nm particles and filaments identical to those found in sera of human patients.

Three different groups introduced cDNA genes from influenza's HA in defective SV40 genomes (Gething and Sambrook, 1981, 1982; Sveda and Lai, 1981; Sveda *et al.*, 1982; Hartman *et al.*, 1982). By immunofluorescence, HA was detected in a Golgi-like perinuclear area and in the plasma membrane (Sveda and Lai, 1981; Sveda *et al.*, 1982; Hartman *et al.*, 1982), where it conferred on the infected cells the ability to agglutinate erythrocytes (Gething and Sambrook, 1981; Sveda *et al.*, 1982). SDS-PAGE analysis of immunoprecipitates showed the presence of a band with the same mobility as mature HA.

Constructs of HA were engineered to study the influence of the signal sequence and of the anchor sequence on the fate of HA. A signal-minus construct coded for a nonglycosylated HA, a result suggesting that this mutant protein was

not translocated across the ER membrane (Gething and Sambrook, 1982). Other constructs were developed lacking segments coding for the C-terminal portion of HA, including the anchor sequence (Sveda *et al.*, 1982; Gething and Sambrook, 1982). These anchor-minus proteins were glycosylated and secreted, although with somewhat lower intracellular transport rates than the complete HA. Some contradictions between the results of these two groups may probably be attributed to differences in the size of the C-terminal deletions.

Rose and Bergmann (1982) microinjected into the nuclei of L cells a complete cDNA gene of the VSV G protein ligated to a plasmid vector, pSV2, under the control of the early SV40 promoter (Mulligan and Berg, 1980). The surface and intracellular immunofluorescence pattern resembled the one described above for HA. An anchor-minus mutant G protein, expressed via a plasmid vector carrying late SV40 promoter sequences, pJC119 (Sprague *et al.*, 1983), introduced by transfection, was secreted from the cells with a half-time of 2–4 hours, much longer than the intracellular transit time of the wild-type G, which reaches the plasma membrane 30 minutes after synthesis (Knipe *et al.*, 1977b; Strous and Lodish, 1980; Bergman *et al.*, 1981). Intending to further define the roles of the anchor sequence and the cytoplasmic domain in the intracellular migration of G, the same investigators engineered a series of mutants with different deletions of the C-terminal segment (Rose and Bergman, 1983). Absence of the cytoplasmic domain or addition of extra amino acids resulted to a failure to exit from the ER or Golgi; deletion of half of the cytoplasmic tail delayed the transport to the surface by about 3 hours.

Influenza virus neuraminidase (NA) is inserted in the membrane by its N-terminal hydrophobic region, the rest of the protein facing the external domain (see Table II; Blok *et al.*, 1982). It possesses two hydrophobic regions: the one at its N-terminus is composed of 29 amino acids and apparently has a dual role of signal peptide and anchor sequence; the other is internal and its function is unknown. Davis *et al.* (1983) have reported expression of a NA cDNA gene ligated to an SV40 defective genome. Immunofluorescence experiments showed staining of perinuclear structures and the cell surface of infected cells.

Kondor-Koch *et al.* (1982) introduced a full length cDNA of the complete genome of Semliki Forest virus (SFV) in pSV2 and microinjected it into the nuclei of BHK cells. Immunofluorescence experiments detected viral capsid protein and viral protein E1 distributed diffusely throughout the cytoplasm, while protein p62 (precursor of the viral membrane glycoproteins E2 and E3) presented the same pattern as described above for glycoproteins of other viruses. By blocking further protein synthesis with cycloheximide, they were able to chase the migration of the immunoreactive product from ER-like to Golgi-like structures and to the cell surface. In further experiments using a modified vector, they obtained synthesis and transport of both E1 and E2 viral proteins to the cell

surface (Kondor-Koch *et al.*, 1983) and were able to show that E1 is responsible for the fusogenic activity of the virion at low pH. They also engineered mutants of E2 glycoprotein (Garoff *et al.*, 1983), deleting different amounts (up to two-thirds) of the cytoplasmic domain, and showed that all of them migrate to the cell surface apparently with the same kinetics as the wild type.

All the results mentioned above confirm that lack of the anchor sequence in a plasma membrane protein results in the eventual secretion of a normally glycosylated smaller protein. These experiments not only demonstrate the membrane attachment role of the anchor sequence, but also strongly indicate that both membrane and secreted forms share the same intracellular migration pathway, at least up to the terminal glycosylation site, the Golgi apparatus. As for the cytoplasmic domain, its role in intracellular migration of plasma membrane glycoproteins is still obscure. Its removal may or may not affect transport, according to the protein. It can be presumed that at least some of the effects described may be related to changes in protein conformation secondary to modifications in the C-terminal end.

Zuniga *et al.* (1983) have engineered mutations in a genomic clone coding for a class I antigen of the mouse major histocompatibility complex. They obtained two mutants lacking different portions of the cytoplasmic domain. When these mutants were introduced into L cells via calcium phosphate, they produced transplantation antigen located in the cell surface, as determined by radioimmunoassay. The antigen was completely functional, as judged by the ability of the cells displaying it to serve as target in specific cytotoxicity assays.

Roth *et al.* (1983) published the first report of polarized expression of influenza's HA coded by a cDNA gene in polarized epithelial cells. They infected primary AGMK cells plated at high density with a recombinant SV40 virus; and in cell patches that presented characteristics of polarized epithelium they observed by immunoelectron microscopy HA inserted preferentially in the apical surface. Because neither the matrix protein nor influenza nucleocapsids were present in the infected cells, this experiment clearly shows that the information directing HA to the apical surface is included in its amino acid sequence. Recently, a rhesus monkey kidney cell line, MA-104, has been shown to generate transepithelial electrical resistances of over 100 Ω cm², to sustain polarized budding of influenza and VSV and to express influenza HA preferentially on the apical surface upon infection with SV40 vectors carrying the HA gene (Gundersen, Roth, Gething, Sambrook, and Rodriguez-Boulan, unpublished observations). Vectors carrying chimeric genes coding for hybrids of apical and basolateral glycoproteins have been generated and are being tested in the MA-104 system for possible changes in the localization of HA that may throw light on the protein domain carrying the information for its apical segregation.

V. SUMMARY AND PERSPECTIVES

Work in recent years has started to unravel the complex mechanisms that eukaryotic cells use to sort and distribute their proteins to the site of function. Two of the "sorting signals" that participate in this process have been identified: the signal sequence, which is employed in translocation across the ER membrane of proteins destined for the different segments of the secretory pathway and for exocytosis, and the mannose 6-P residues of lysosomal proteins, which are responsible for their targeting to lysosomes. Substantial progress has been made in the characterization of the receptors for these two sorting signals. However, very little is known about the mechanisms that mediate the localization and concentration of specific proteins and lipids within organelles. Various experimental model systems have become available for their study. The advent of recombinant DNA technology has shortened the time needed for obtaining the primary structure of proteins to a few months. Consequently, there are now several membrane proteins, including some receptors, the primary sequences of which are known, and the list is increasing every month. This information, in combination with new and more efficient methods to reintroduce and express genes into cells, makes it possible to plan strategies to identify the protein domains involved in sorting; these, eventually, could be used to identify the "sorters" themselves. The ultimate knowledge of the mechanisms that the cell uses to distribute its components is not only an important aspect of cell biology but may also bear significant practical relevance for the treatment of some genetic and oncogenic disorders.

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REFERENCES

- Abrahamson, D. R., and Rodewald, R. (1981). Evidence of the sorting of endocytic vesicle contents during the receptor-mediated transport of IgG across the newborn rat intestine. *J. Cell Biol.* **91**, 270-280.
- Adams, W. R., and Kraft, M. L. (1967). Electron microscopic study of the intestinal epithelium of mice infected with the agent of epizootic diarrhea of infant mice. *Am. J. Pathol.* **51**, 39-60.
- Alonso, F. V., and Compans, R. W. (1981). Differential effect of monensin on enveloped viruses that form at distinct plasma membrane domains. *J. Cell Biol.* **89**, 700-705.
- Altstiel, L., and Branton, D. (1983). Fusion of coated vesicles with lysosomes: Measurement with a fluorescence assay. *Cell* **32**, 921-929.

- Anderson, G. W., and Kaplan, J. (1983). Receptor-mediated endocytosis. In "Modern Cell Biology" (B. Satir, ed.), Vol. 1, pp. 1-52. Liss, New York.
- Bar-Nun, S., Kreibich, G., Adesnik, M., Alterman, L., Negishi, M., and Sabatini, D. D. (1980). Synthesis and insertion of cytochrome P-450 into endoplasmic reticulum membranes. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 965-969.
- Bell, R. M., and Coleman, R. A. (1980). Enzymes of glycerolipid synthesis in eukaryotes. *Annu. Rev. Biochem.* **49**, 459-488.
- Bergeron, J. J. M., Kotwal, G. J., Levine, G., Bilan, P., Rachubinsky, R., Hamilton, M., Shore, G. C., and Ghosh, H. P. (1982). Intracellular transport of the glycoprotein G of vesicular stomatitis virus as visualized by electron microscope radioautography. *J. Cell Biol.* **94**, 36-41.
- Bergmann, J. E., Tokuyasu, K. T., and Singer, S. J. (1981). Passage of an integral membrane protein, the vesicular stomatitis virus glycoprotein through the Golgi apparatus en route to the plasma membrane. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1746-1750.
- Blobel, G. (1980). Intracellular protein topogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1496-1500.
- Blobel, G., and Dobberstein, B. (1975a). Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* **67**, 835-851.
- Blobel, G., and Dobberstein, B. (1975b). Transfer of proteins across membranes. II. Reconstitution of functional rough microsomes from heterologous components. *J. Cell Biol.* **67**, 852-862.
- Blobel, G., and Sabatini, D. D. (1971). Ribosome-membrane interaction in eukaryotic cells. In "Biomembranes" (L. A. Manson, ed.), Vol. 2, pp. 193-195. Plenum, New York.
- Blobel, G., Walter, P., Chang, C. N., Goldman, B., Erickson, A. H., and Lingappa, V. R. (1979). Translocation of proteins across membranes: The signal hypothesis and beyond. *Symp. Soc. Exp. Biol.* **33**, 9-36.
- Blok, J., Air, G. M., Laver, W. G., Ward, C. W., Lilley, G. G., Woods, E. F., Roxburgh, C. M., and Inglis, A. S. (1982). Studies on the size, chemical composition, and partial sequence of the neuraminidase (NA) from type A influenza viruses show that the N-terminal region of the NA is not processed and serves to anchor the NA in the viral membrane. *Virology* **119**, 109-121.
- Boquet, P., and Duflot, E. (1982). Tetanus toxin fragment forms channels in lipid vesicles at low pH. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7614-7618.
- Borgese, N., and Gaetani, S. (1980). Site of synthesis of rat liver NADH cytochrome b5 reductase, an integral membrane protein. *FEBS Lett.* **112**, 216-220.
- Braell, W. A., and Lodish, H. F. (1982). The erythrocyte anion transport protein is cotranslationally inserted into microsomes. *Cell* **28**, 23-31.
- Branton, D., Cohen, C. M., and Tyler, J. (1981). Interactions of cytoskeletal proteins on the human erythrocyte membrane. *Cell* **24**, 24-32.
- Bretscher, M. S. (1973). Membrane structure: Some general principles. *Science* **181**, 622-629.
- Bretscher, M. S. (1981). Surface uptake by fibroblasts and its consequences. *Cold Spring Harbor Symp. Quant. Biol.* **46**, 707-712.
- Brown, M. S., and Goldstein, J. L. (1979). Receptor-mediated endocytosis: Insights from the lipoprotein receptor system. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3330-3337.
- Brown, M. S., Anderson, R. G. W., and Goldstein, J. L. (1983). Recycling receptors: The round-trip itinerary of migrant membrane proteins. *Cell* **32**, 663-667.
- Brown, W. J., and Farquhar, M. C. (1984). The mannose-6-phosphate receptor is concentrated in cis Golgi cisternae. *Cell* **36**, 295-307.
- Brunner, J., Hauser, H., Braun, H., Wilson, K. J., Wacker, H., O'Neill, B., and Semenza, G. (1979). The mode of association of the enzyme complex sucrose-isomaltase with the intestinal brush border membrane. *J. Biol. Chem.* **254**, 1821-1828.
- Cerejido, M., Robbins, E. S., Dolan, W. J., Rotunno, C. A., and Sabatini, D. D. (1978). Polarized

- monolayers formed by epithelial cells on a permeable and translucent support. *J. Cell Biol.* **77**, 853–880.
- Chambard, M., Gabrion, J., and Mauchamp, J. (1981). Influence of collagen gel on the orientation of epithelial cell polarity: Follicle formation from isolated thyroid cells and from preformed monolayers. *J. Cell Biol.* **91**, 157–166.
- Chua, N.-H., and Schmidt, G. W. (1979). Transport of proteins into mitochondria and chloroplasts. *J. Cell Biol.* **81**, 461–483.
- Claudio, T., Ballivet, M., Patrick, J., and Heineman, S. (1983). Nucleotide and deduced amino acid sequences of *Torpedo californica* acetylcholine receptor gamma subunit. *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1111–1115.
- Colman, P. M., Varghese, J. N., and Laver, W. G. (1983). Structure of the catalytic and antigenic sites of influenza virus neuraminidase. *Nature (London)* **303**, 41–44.
- Davies, P. J. A., Davies, D. R., Levitzky, A., Maxfield, F. R., Milhaud, P., Willingham, M. C., and Pastan, I. (1980). Transglutaminase is essential in receptor mediated endocytosis of alpha-2-macroglobulin and polypeptide hormones. *Nature (London)* **283**, 162–167.
- Davis, A. R., Bos, J. T., and Nayak, D. P. (1983). Active influenza virus neuraminidase is expressed in monkey cells from cDNA cloned in simian virus 40 vectors. *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3976–3980.
- Davis, B. P., and Tai, P.-C. (1980). The mechanism of protein secretion across membranes. *Nature (London)* **283**, 433–438.
- Dobberstein, B., Blobel, G., and Chua, N.-H. (1977). In vitro synthesis and processing of a putative precursor for the small subunit of ribulose-1,5-bisphosphate carboxylase of *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 343–347.
- Dobberstein, B., Garoff, H., Warren, G., and Robinson, P. J. (1979). Cell free synthesis and membrane insertion of mouse H2d histocompatibility antigen and beta 2-microglobulin. *Cell* **17**, 759–769.
- Donovan, J. J., Simon, M. I., Draper, R. K., and Montal, M. (1981). Diphtheria toxin forms transmembrane channels in planar lipid bilayers. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 172–176.
- Dunn, W. A., Hubbard, A. L., and Aronson, Jr., N. N. (1980). Low temperature selectively inhibits fusion between pinocytic vesicles and lysosomes during heterophagy of ¹²⁵I-asialofetuin by the perfused rat liver. *J. Biol. Chem.* **255**, 5971–5978.
- Dunphy, W. G., and Rothman, J. E. (1983). Compartmentation of asparagine-linked oligosaccharide processing in the Golgi apparatus. *J. Cell Biol.* **97**, 270–275.
- Early, P., Rogers, J., Davis, M., Calame, K., Bond, M., Wall, R., and Hood, L. (1980). Two mRNAs can be produced from a single immunoglobulin μ gene by alternative RNA processing pathways. *Cell* **20**, 313–319.
- Ehrenreich, J. H., Bergeron, J. J. M., Siekivitz, P., and Palade, G. E. (1973). Golgi fractions prepared from rat liver homogenates. I. Isolation procedure and morphological characterization. *J. Cell Biol.* **59**, 45–72.
- Elder, J. T., Spritz, R. A., and Weissman, S. M. (1981). Simian virus 40 as an eukaryotic cloning vehicle. *Annu. Rev. Genet.* **15**, 295–340.
- Emr, S. D., Hall, M. N., and Silhavy, T. J. (1980). A mechanism of protein localization: The signal hypothesis and bacteria. *J. Cell Biol.* **86**, 701–711.
- Erickson, A. H., and Blobel, G. (1979). Early events in the biosynthesis of the lysosomal enzyme cathepsin D. *J. Biol. Chem.* **254**, 11771–11774.
- Esmon, B., Novick, P., and Schekman, R. (1980). Compartmentalized assembly of oligosaccharides on exported glycoproteins in yeast. *Cell* **25**, 451–460.
- Evans, G. A., Margulies, D. H., Camerini-Otero, R. D., Ozato, K., and Seidman, J. G. (1982). Structure and expression of a mouse histocompatibility antigen gene, H-2Ld. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1994–1998.

- Farquhar, M. G. (1982). Membrane recycling in secretory cells: Pathway to the Golgi complex. *Ciba Found. Symp.* **92**, 157–183.
- Farquhar, M. G. (1983). Multiple pathways of exocytosis, endocytosis, and membrane recycling: Validation of a Golgi route. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **42**, 2407–2413.
- Farquhar, M., and Palade, G. (1981). The Golgi apparatus (complex)-(1954–1981)-from artifact to center stage. *J. Cell Biol.* **91**, 77s–103s.
- Ferro-Novick, S., Hansen, W., Schauer, I., and Scheckman, R. (1984a). Genes required for completion of import of proteins into the endoplasmic reticulum in yeast. *J. Cell Biol.* **98**, 44–53.
- Ferro-Novick, S., Novick, P., Field, C., and Schekman, R. (1984b). Yeast secretory mutants that block the formation of active cell surface enzymes. *J. Cell Biol.* **98**, 35–43.
- Field, C., and Schekman, R. (1980). Localized secretion of acid phosphatase reflects the pattern of cell surface growth in *Saccharomyces cerevisiae*. *J. Cell Biol.* **86**, 123–128.
- Fields, S., Winter, G., and Brownlee, G. G. (1981). Structure of the neuraminidase gene in human influenza virus A/PR/8/34. *Nature (London)* **290**, 213–217.
- Fitting, T., and Kabat, D. (1982). Evidence for a glycoprotein “signal” involved in transport between subcellular organelles: Two membrane glycoproteins reach the cell surface at different rates. *J. Biol. Chem.* **257**, 14011–14017.
- Forgac, M., Cantley, L., Wiedenmann, B., Altstiel, L., and Branton, D. (1983). Clathrin coated vesicles contain an ATP-dependent proton pump. *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1300–1303.
- Frank, G., Brunner, J., Hauser, H., Wacker, H., Semenza, G., and Zuber, H. (1978). The hydrophobic anchor of small intestinal sucrase-isomaltase. *FEBS Lett.* **96**, 183–188.
- Friend, D. S., and Farquhar, M. G. (1967). Functions of coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol.* **35**, 357–376.
- Fries, D. S., and Rothman, J. E. (1980). Transport of vesicular stomatitis virus glycoprotein in a cell free extract. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3870–3874.
- Fries, E., and Rothman, J. E. (1981). Transient activity of Golgi-like membranes as donors of vesicular stomatitis viral glycoprotein *in vitro*. *J. Cell Biol.* **90**, 697–704.
- Furthmayr, H. (1977). Structural analysis of a membrane glycoprotein: Glycophorin A. *J. Supramol. Struct.* **7**, 121–134.
- Garoff, H., and Soderlund, H. (1978). The amphiphilic membrane glycoproteins of Semliki Forest virus are attached to the lipid bilayer by their COOH-terminal ends. *J. Mol. Biol.* **124**, 535–549.
- Garoff, H., Simons, K., and Dobberstein, B. (1978). Assembly of the Semliki Forest virus membrane glycoproteins in the membrane of the endoplasmic reticulum *in vitro*. *J. Mol. Biol.* **124**, 587–600.
- Garoff, H., Frischauf, A.-M., Simons, K., Lehrach, H., and Delius, H. (1980). Nucleotide sequence of cDNA coding for Semliki forest virus membrane glycoproteins. *Nature (London)* **288**, 236–241.
- Garoff, H., Kondor-Koch, C., Petterson, R., and Burke, R. (1983). Expression of Semliki Forest virus proteins from cloned complementary DNA. II. The membrane-spanning glycoprotein E2 is transported to the cell surface without its normal cytoplasmic domain. *J. Cell Biol.* **97**, 652–658.
- Gething, M. J., and Sambrook, J. (1981). Cell-surface expression of influenza hemagglutinin from a cloned DNA copy of the RNA gene. *Nature (London)* **292**, 620–625.
- Gething, M. J., and Sambrook, J. (1982). Construction of influenza hemagglutinin genes that code for intracellular and secreted forms of the protein. *Nature (London)* **300**, 598–603.
- Gething, M. J., Bye, J., Skehel, J., and Waterfield, M. (1980). Cloning and DNA sequence of double stranded copies of hemagglutinin from H2 and H3 strains elucidates antigenic shift and drift in human influenza virus. *Nature (London)* **287**, 301–306.
- Geuze, H. J., Slot, J. W., Strouss, G. J. A. M., Lodish, H. F., and Schwartz, A. L. (1983).

- Intracellular site of asialoglycoprotein receptor-ligand uncoupling: Double-label immunoelectron microscopy during receptor-mediated endocytosis. *Cell* **32**, 277-287.
- Gibson, R., Leavitt, R., Kornfeld, S., and Schlesinger, S. (1978). Synthesis and infectivity of vesicular stomatitis virus containing non-glycosylated G protein. *Cell* **13**, 671-679.
- Gilmore, R., Blobel, G., and Walter, P. (1982a). Protein translocation across the endoplasmic reticulum. I. Detection in the microsomal membrane of a receptor for the signal recognition particle. *J. Cell Biol.* **95**, 463-469.
- Gilmore, R., Walter, P., and Blobel, G. (1982b). Protein translocation across the endoplasmic reticulum. II. Isolation and characterization of the signal recognition particle receptor. *J. Cell Biol.* **95**, 470-477.
- Glickman, J., Croen, K., Kelly, S., and Al-Awqati, Q. (1983). Golgi membranes contain an electrogenic H⁺ pump parallel to a chloride conductance. *J. Cell Biol.* **97**, 1303-1308.
- Gluzman, Y., ed. (1982). "Eukaryotic Viral Vectors." Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Goldman, B. M., and Blobel, G. (1978). Biogenesis of peroxysomes: Intracellular site of synthesis of catalase and uricase. *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5066-5070.
- Goldstein, J. L., Anderson, R. G. W., and Brown, M. S. (1979). Coated pits, coated vesicles, and receptor-mediated endocytosis. *Nature (London)* **279**, 679-685.
- Gonzalez-Noriega, A., Grubb, J. H., Talkad, V., and Sly, W. S. (1980). Chloroquine inhibits lysosomal enzyme pinocytosis and enhances lysosomal enzyme secretion by impairing receptor recycling. *J. Cell Biol.* **85**, 839-852.
- Green, J., Griffiths, G., Louvard, D., Quinn, P., and Warren, G. (1981). Passage of viral membrane proteins through the Golgi complex. *J. Mol. Biol.* **152**, 663-698.
- Green, R. F., Meiss, H. K., and Rodriguez-Boulán, E. (1981). Glycosylation does not determine segregation of viral glycoproteins in the plasma membrane of epithelial cells. *J. Cell Biol.* **89**, 230-239.
- Griffiths, G., Brands, R., Burke, B., Louvard, D., and Warren, G. (1982). Viral membrane proteins acquire galactose in trans Golgi cisternae during intracellular transport. *J. Cell Biol.* **95**, 781-792.
- Griffiths, G., Quinn, P., and Warren, G. (1983). Dissection of the Golgi complex. I. Monensin inhibits the transport of viral membrane proteins from medial to trans Golgi cisternae in baby hamster kidney cell infected with Semliki Forest virus. *J. Cell Biol.* **96**, 835-850.
- Gumbiner, B., and Kelly, R. B. (1982). Two distinct intracellular pathways transport secretory and membrane glycoproteins to the surface of pituitary tumor cells. *Cell* **28**, 51-59.
- Hartman, J. R., Nayak, D. P., and Fareed, G. C. (1982). Human influenza virus hemagglutinin is expressed in monkey cells using simian virus 40 vectors. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 233-237.
- Hasilik, A., and Neufeld, E. (1980a). Biosynthesis of lysosomal enzymes in fibroblasts—synthesis as precursors of higher molecular weight. *J. Biol. Chem.* **255**, 4937-4945.
- Hasilik, A., and Neufeld, E. (1980b). Biosynthesis of lysosomal enzymes in fibroblasts—phosphorylation of mannose residues. *J. Biol. Chem.* **255**, 4946-4950.
- Hasilik, A., and Tanner, W. (1978). Carbohydrate moieties of carboxypeptidase Y and perturbation of its biosynthesis. *Eur. J. Biochem.* **91**, 567-571.
- Hasilik, A., Waheed, A., and von Figura, K. (1981). Enzymatic phosphorylation of lysosomal enzymes in the presence of UDP-N-acetylglucosamine: Absence of activity in I-cell fibroblasts. *Biochem. Biophys. Res. Commun.* **98**, 761-767.
- Hauri, H. P., Quaroni, A., and Isselbacher, K. J. (1979). Biosynthesis of intestinal plasma membrane: Post-translational route and cleavage of sucrase-isomaltase. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5183-5186.
- Hauri, H. P., Wacker, H., Rickli, E. E., Bigler-Meier, B., Quaroni, A., and Semenza, G. (1982).

- Biosynthesis of sucrase isomaltase. Purification and NH₂-terminal amino acid sequence of the rat sucrase isomaltase precursor (pro-sucrase-isomaltase) from fetal intestinal transplants. *J. Biol. Chem.* **257**, 4522–4528.
- Helenius, A., Mellman, I., Wall, D., and Hubbard, A. (1983). Endosomes. *Trends Biochem. Sci.* **8**, 245–250.
- Herzog, V., and Farquhar, M. G. (1977). Luminal membrane retrieved after exocytosis reaches most Golgi cisternae in secretory cells. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5073–5077.
- Herzog, V., and Miller, F. (1979). Membrane retrieval in epithelial cells of isolated thyroid follicles. *Eur. J. Cell Biol.* **19**, 203–215.
- Hickman, S., and Neufeld, E. F. (1972). A hypothesis for I-cell disease: Defective hydrolases that do not enter the lysosomes. *Biochem. Biophys. Res. Commun.* **49**, 992–999.
- Hirano, H., Parkhouse, B., Nicolson, G. L., Lennox, E. S., and Singer, J. S. (1972). Distribution of saccharide residues on membrane fragments from a myeloma-cell homogenate: Its implication membrane biogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2945–2949.
- Hiti, A. L., Davis, A. R., and Nayak, D. P. (1981). Complete sequence analysis shows that the hemagglutinins of the H0 and H2 subtypes of human influenza are closely related. *Virology* **111**, 113–124.
- Holmes, I. H. (1983). Rotaviruses. In "The Reoviridae" (W. K. Joklik, ed.), pp. 359–423. Plenum, New York.
- Hubbard, S. C., and Ivatt, R. J. (1981). Synthesis and processing of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **50**, 555–583.
- Hunt, L. A., Etchison, J. A., and Summers, D. F. (1978). Oligosaccharide chains are trimmed during synthesis of the envelope glycoprotein of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. U.S.A.* **75**, 754–758.
- Ito, A., and Palade, G. E. (1978). Presence of NADPH-cytochrome P-450 reductase in rat liver Golgi membranes. *J. Cell Biol.* **79**, 590–597.
- Jamieson, J. D., and Palade, G. (1971). Synthesis, intracellular transport and discharge of secretory proteins in stimulated exocrine cells. *J. Cell Biol.* **50**, 135–158.
- Jamieson, J. D., and Palade, G. E. (1977). Production of secretory proteins in animal cells. *Int. Cell Biol.*, pp. 308–317.
- Jelsema, C. L., and Morre, D. J. (1978). Distribution of phospholipid biosynthetic enzymes among cell components of rat liver. *J. Biol. Chem.* **253**, 7960–7971.
- Johnson, D. C., and Schlesinger, M. J. (1980). Vesicular stomatitis virus and Sindbis virus glycoprotein transport to the cell surface is inhibited by ionophores. *Virology* **103**, 407–424.
- Jokinen, M., Gahmberg, C. G., and Anderson, L. A. (1979). Biosynthesis of the major human red cell sialoglycoprotein, glycophorin A, in a continuous cell line. *Nature (London)* **279**, 604–607.
- Kaariainen, L., Hashimoto, K., Saraste, J., Virtanen, I., and Penttinen, K. (1980). Monensin and FCCP inhibit the intracellular transport of alphavirus membrane glycoproteins. *J. Cell Biol.* **87**, 783–791.
- Katz, F. N., Rothman, J. E., Knipe, D. M., and Lodish, H. F. (1977). Membrane assembly: Synthesis and intracellular processing of the vesicular stomatitis glycoprotein. *J. Supramol. Struct.* **7**, 353–370.
- Kehry, M., Ewald, S., Douglas, R., Sibley, C., Raschke, W., Fambrough, D., and Hood, L. (1980). The immunoglobulin μ chains of membrane-bound and secreted IgM molecules differ in their C-terminal segments. *Cell* **21**, 393–406.
- Kenny, A. J., and Maroux, S. (1982). Topology of microvillar membrane hydrolases of kidney and intestine. *Physiol. Rev.* **62**, 91–128.
- Knipe, D. M., Baltimore, D., and Lodish, H. F. (1977a). Maturation of viral proteins in cells infected with temperature-sensitive mutants of vesicular stomatitis virus. *J. Virol.* **21**, 1149–1158.

- Knipe, D. M., Lodish, H. F., and Baltimore, D. (1977b). Localization of two cellular forms of the vesicular stomatitis virus glycoprotein. *J. Virol.* **21**, 1121–1127.
- Kondor-Koch, C., Riedel, H., Soderberg, K., and Garoff, H. (1982). Expression of the structural protein of Semliki forest virus from cloned cDNA microinjected into the nucleus of baby hamster kidney cells. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4525–4529.
- Kondor-Koch, C., Burke, B., and Garoff, H. (1983). Expression of Semliki Forest virus proteins from cloned complementary DNA. I. Fusion activity of the spike glycoprotein. *J. Cell Biol.* **97**, 644–651.
- Krachenbuhl, J. P., and Kuhn, L. (1978). Transport of immunoglobulins across epithelia. In "Transport of macromolecules in cellular systems" (S. C. Silverstein, ed.), pp. 213–228. Dahlem Konferenzen, Berlin.
- Krangel, M. S., Orr, H. T., and Strominger, J. L. (1979). Assembly and maturation of HLA-A and HLA-B *in vivo*. *Cell* **18**, 979–991.
- Kreibich, G., Czako-Graham, M., Grebenau, R., Mok, W., Rodriguez-Boulán, E., and Sabatini, D. D. (1978a). Characterization of the ribosomal binding site in rat liver rough microsomes: Ribophorins I and II, two integral membrane proteins related to ribosome binding. *J. Supramol. Struct.* **8**, 279–302.
- Kreibich, G., Freinstein, C. M., Pereyra, B. N., Ulrich, B. L., and Sabatini, D. D. (1978b). Proteins of rough microsomal membranes related to ribosome binding. II. Cross-linking of bound ribosomes to specific membrane proteins exposed at the binding sites. *J. Cell Biol.* **77**, 488–506.
- Kreibich, G., Ulrich, B. L., and Sabatini, D. D. (1978c). Proteins of rough microsomal membranes related to ribosome binding. I. Identification of ribophorins I and II, membrane proteins characteristic of rough microsomes. *J. Cell Biol.* **77**, 464–487.
- Kreil, G. (1981). Transfer of proteins across membranes. *Annu. Rev. Biochem.* **50**, 317–348.
- Lefay, F. (1974). Envelope proteins of vesicular stomatitis virus: Effects of temperature sensitive mutations in complementation groups III and V. *J. Virol.* **14**, 1220–1228.
- Lazarow, P. (1980). Functions and biosynthesis of lysosomes. *Int. Cell Biol.* pp. 633–639.
- Lazarowitz, S. G., and Choppin, P. W. (1975). Enhancement of the infectivity of influenza A and B viruses by proteolytic cleavage of the hemagglutinin polypeptide. *Virology* **68**, 440–454.
- Leblond, C. P., and Bennett, G. (1977). Role of the Golgi apparatus in terminal glycosylation. *Int. Cell Biol.* pp. 326–336.
- Ledford, B. E., and Davis, D. F. (1983). Kinetics of serum protein secretion by cultured hepatoma cells: Evidence for multiple secretory pathways. *J. Biol. Chem.* **258**, 3304–3308.
- Lehle, L., Cohen, R. E., and Ballou, C. E. (1979). Carbohydrate structure of yeast invertase. *J. Biol. Chem.* **254**, 12209–12218.
- Lenard, J. (1978). Virus envelopes and plasma membranes. *Annu. Rev. Biophys. Bioeng.* **7**, 139–165.
- Lenard, J., and Compans, R. W. (1974). The membrane structure of lipid-containing viruses. *Biochim. Biophys. Acta* **344**, 51–94.
- Lennarz, W. J. (1980). In "The Biochemistry of Glycoproteins and Proteoglycans" (W. J. Lennarz, ed.), pp. 35–84. Plenum, New York.
- Lingappa, V. R., Katz, F. N., Lodish, H. F., and Blobel, G. (1978). A signal sequence for the insertion of a transmembrane glycoprotein. Similarities to the signals of secretory proteins in primary structure and function. *J. Biol. Chem.* **253**, 8667–8670.
- Lodish, H. F., and Kong, N. (1984). Glucose removal from N-linked oligosaccharides is required for efficient maturation of certain secretory glycoproteins from the rough endoplasmic reticulum to the Golgi complex. *J. Cell Biol.* **98**, 1720–1729.
- Lodish, H. F., Braell, W. A., Schwartz, A. L., Strous, G. J. A. M., and Zilberstein, A. (1981). Synthesis and assembly of membrane and organelle proteins. *Int. Rev. Cytol. Suppl.* **12**, 247–307.

- Lodish, H. F., Kong, N., Snider, M., and Strouss, G. J. A. M. (1983). Hepatoma secretory proteins migrate from the rough endoplasmic reticulum to the Golgi at characteristic rates. *Nature (London)* **304**, 80–83.
- Lohmeyer, J., and Klenk, H. D. (1979). A mutant of influenza virus with a temperature sensitive defect in the posttranslational processing of the hemagglutinin. *Virology* **93**, 134–145.
- Lojda, Z. (1974). Cytochemistry of enterocytes and of other cells in the mucous membrane of the small intestine. *Biomembranes* **4A**, 43–122.
- Louvard, D., Reggio, H., and Warren, G. (1982). Antibodies to the Golgi complex and rough endoplasmic reticulum. *J. Cell Biol.* **92**, 92–107.
- Lusis, A. J., and Swank, R. T. (1980). Regulation of location of intracellular proteins. In "Cell Biology, a Comprehensive Treatise" (D. M. Prescott and L. Goldstein, eds.), Vol. 4, pp. 339–391. Academic Press, New York.
- McKusick, V. A., Neufeld, E. F., and Kelly, T. T. (1972). Two disorders of lysosomal enzyme localization mucopolipidosis II (ML II, I-cell disease, inclusion cell disease and mucopolipidosis III (MLIII), pseudo-Hurler polydystrophy). In "The Metabolic Basis of Inherited Disease" (J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, eds.), pp. 1299–1302. McGraw Hill, New York.
- Marchesi, V. T., Furthmayr, H., and Tomita, M. (1976). The red cell membrane. *Annu. Rev. Biochem.* **45**, 667–698.
- Marsh, M., Bolzau, E., and Helenius, A. (1983). Penetration of Semliki Forest Virus from acidic prelysosomal vacuoles. *Cell* **32**, 931–940.
- Matlin, A., and Simons, K. (1983). Reduced temperature prevents transfer of a membrane glycoprotein to the cell surface but not terminal glycosylation. *Cell* **34**, 233–243.
- Matlin, K., Bainton, D. F., Pesonen, M., Louvard, D., Genty, N., and Simons, K. (1983). Transepithelial transport of a viral membrane glycoprotein implanted into the apical plasma membrane of Madin–Darby Canine Kidney cells. I. Morphological evidence. *J. Cell Biol.* **97**, 627–637.
- Maxfield, F. R. (1982). Weak bases and ionophores rapidly and reversibly raise the pH of endocytic vesicles in cultured mouse fibroblasts. *J. Cell Biol.* **95**, 676–681.
- Meiss, H. K., Green, R., and Rodriguez-Boulan, E. J. (1982). Lectin resistant mutants of polarized epithelial cells. *Mol. Cell. Biol.* **2**, 1287–1294.
- Meyer, D. I., and Dobberstein, B. (1980a). A membrane component essential for vectorial translocation of nascent proteins across the endoplasmic reticulum: Requirement for its extraction and reassociation with the membrane. *J. Cell Biol.* **87**, 498–502.
- Meyer, D. I., and Dobberstein, B. (1980b). Identification and characterization of a membrane component essential for the translocation of nascent proteins across the membrane of the endoplasmic reticulum. *J. Cell Biol.* **87**, 503–508.
- Meyer, D. I., Krause, E., and Dobberstein, B. (1982). Secretory protein translocation across membranes—the role of the "docking protein." *Nature (London)* **297**, 647–650.
- Milstein, C., Brownlee, G. G., Harrison, T. M., and Mathews, M. B. (1972). A possible precursor of immunoglobulin light chains. *Nature (London) New Biol.* **239**, 117–120.
- Min Jou, W., Verhoeyen, M., Devos, R., Saman, E., Fang, R., Huylebroeck, D., Fiers, W., Threlfall, G., Barber, C., Carey, N., and Emtage, S. (1980). Complete structure of the hemagglutinin gene from the human influenza A/Victoria/3/75 (H3N2) strain as determined from cloned DNA. *Cell* **19**, 683–696.
- Misek, D. E., Bard, E., and Rodriguez-Boulan, E. (1984). Biogenesis of epithelial cell polarity. Intracellular sorting and vectorial exocytosis of an apical plasma membrane glycoprotein. *Cell*, **39**, 537–546.
- Misfeldt, D. S., Hamamoto, S. T., and Pitelka, D. R. (1976). Transepithelial transport in cell culture. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1212–1216.
- Moore, H.-P. Gumbiner, B., and Kelly, R. B. (1983a). A subclass of proteins and sulfated mac-

- romolecules secreted by AtT-20 (mouse pituitary tumor) cells is sorted with adrenocorticotropin into dense secretory granules. *J. Cell Biol.* **97**, 810–817.
- Moore, H.-P., Gumbiner, B., and Kelly, R. B. (1983b). Chloroquine diverts ACTH from a regulated to a constitutive secretory pathway in AtT-20 cells. *Nature (London)* **302**, 434–436.
- Moriarty, A. M., Hoyer, B. H., Shih, J. W. K., Gehrin, J. L., and Hamer, D. H. (1981). Expression of the hepatitis B virus surface antigen gene in cell culture by using a simian virus 40 vector. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2606–2610.
- Mostov, K. E., Friedlander, M., and Blobel, G. (1984). The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. *Nature (London)* **308**, 37–43.
- Mudd, J. A. (1974). Glycoprotein fragment associated with vesicular stomatitis virus after proteolytic digestion. *Virology* **62**, 573–577.
- Mulligan, R. C., and Berg, P. (1980). Expression of a bacterial gene in mammalian cells. *Science* **209**, 1422–1427.
- Nakamura, K., and Compans, R. W. (1977). The cellular site of sulfation of influenza viral glycoproteins. *Virology* **79**, 381–392.
- Neufeld, E. F., Sando, G. N., Garvin, A. J., and Rome, L. H. (1977). The transport of lysosomal enzymes. *J. Supramol. Struct.* **6**, 95–101.
- Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Furutani, Y., Hirose, T., Asai, M., Inayama, S., Miyata, T., and Numa, S. (1982). Primary structure of alpha subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequence. *Nature (London)* **299**, 793–797.
- Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Tanabe, T., Shimizu, S., Kikuyotani, S., Kayano, T., Hirose, T., Inayama, S., and Numa, S. (1983a). Cloning and sequence analysis of calf cDNA and human genomic DNA encoding α -subunit precursor of muscle acetylcholine receptor. *Nature (London)* **305**, 818–823.
- Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikuyotani, S., Furutani, Y., Hirose, T., Takashima, H., Inayama, S., Miyata, T., and Numa, S. (1983b). Structural homology of *Torpedo californica* acetylcholine receptor subunits. *Nature (London)* **302**, 528–532.
- Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikuyotani, S., Hirose, T., Asai, M., Takashima, H., Inayama, S., Miyata, T., and Numa, S. (1983c). Primary structures of beta and delta subunit precursors of *Torpedo californica* acetylcholine receptor deduced from cDNA sequences. *Nature (London)* **301**, 251–255.
- Novick, P., and Schekman, R. (1979). Secretion and cell surface growth are blocked in a temperature-sensitive mutant of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1858–1862.
- Novick, P., and Schekman, R. (1983). Export of major cell surface proteins is blocked in yeast secretory mutants. *J. Cell Biol.* **96**, 541–547.
- Novick, P., Field, C., and Schekman, R. (1980). Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* **21**, 205–215.
- Novick, P., Ferro, S., and Schekman, R. (1981). Order of events in the yeast secretory pathway. *Cell* **25**, 461–469.
- Novikoff, A. B. (1976). The endoplasmic reticulum: A cytochemist's view (a review). *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2781–2787.
- Novikoff, A. B., and Novikoff, P. M. (1977). Cytochemical contribution to differentiating GERL from Golgi apparatus. *Histochem. J.* **9**, 525–552.
- Novikoff, P. M., Novikoff, A. B., Quintana, N., and Hauw, J. J. (1971). Golgi apparatus, GERL, and lysosomes of neurons in rat dorsal root ganglia, studied by thick section and thin section cytochemistry. *J. Cell Biol.* **50**, 859–886.
- Onishi, H. R., Tkacz, J. S., and Lampen, J. O. (1979). Glycoprotein nature of yeast alkaline phosphatase. Formation of an active enzyme in the presence of tunicamycin. *J. Biol. Chem.* **254**, 11943–11952.

- Op den Kamp, J. A. F. (1979). Lipid asymmetry in membranes. *Annu. Rev. Biochem.* **48**, 47–71.
- Owen, M. J., Kissonergis, A. M., Lodish, H. F., and Crumpton, M. J. (1981). Biosynthesis and maturation of HLA-DR antigens *in vivo*. *J. Biol. Chem.* **256**, 8887–8893.
- Pagano, R. E., and Longmuir, K. J. (1983). Intracellular translocation and metabolism of fluorescent lipid analogues in cultured mammalian cells. *Trends Biochem. Sci.* **8**, 157–161.
- Palade, G. (1975). Intracellular aspects of the process of protein secretion. *Science* **189**, 347–358.
- Pappenheimer, A. M., Jr. (1978). Diphtheria: Molecular biology of an infectious process. *Trends Biochem. Sci.* **3**, N220–N224.
- Parham, P., Alpert, B. N., Orr, H. T., and Strominger, J. L. (1977). Carbohydrate moiety of HLA antigens. Antigenic properties and amino acid sequences around the site of glycosylation. *J. Biol. Chem.* **252**, 7555–7567.
- Parodi, A. J., and Leloir, L. F. (1979). The role of lipid intermediates in the glycosylation of proteins in the eukaryotic cell. *Biochim. Biophys. Acta* **559**, 1–37.
- Pastan, I., and Willingham, M. C. (1983). Receptor mediated endocytosis, coated pits, receptors and the Golgi. *Trends Biochem. Sci.* **8**, 250–254.
- Pearson, G. R., and McNulty, M. S. (1979). Ultrastructural changes in small intestinal epithelium of neonatal pigs infected with pig rotavirus. *Arch. Virol.* **59**, 127–136.
- Pease, L. R., Nathenson, S. G., and Leinward, L. A. (1982). Mapping class I gene sequences in the major histocompatibility complex. *Nature (London)* **298**, 382–385.
- Pesonen, M., and Simons, K. (1983). Transepithelial transport of a viral membrane glycoprotein implanted into the apical plasma membrane of Madin–Darby Canine kidney cells. II. Immunological quantitation. *J. Cell Biol.* **97**, 638–643.
- Pisam, M., and Ripoche, P. (1976). Redistribution of surface macromolecules in dissociated epithelial cells. *J. Cell Biol.* **71**, 907–920.
- Ploegh, H. L., Cannon, L. E., and Strominger, J. L. (1979). Cell free translation of the mRNAs for the heavy and light chains of HLA-A and HLA-B antigens. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2273–2277.
- Ploegh, H. L., Orr, H. T., and Strominger, J. L. (1981). Major histocompatibility antigens: The human (HLA-A, -B, -C) and murine (H-2K, H-2D) class I molecules. *Cell* **24**, 287–299.
- Pollack, L., and Atkinson, P. H. (1983). Correlation of glycosylation forms with position in amino acid sequence. *J. Cell Biol.* **97**, 293–300.
- Porter, A. G., Carber, C., Carey, N. H., Hallewell, R. A., Threlfall, G., and Emtage, J. S. (1979). Complete nucleotide sequence of an influenza virus hemagglutinin gene from cloned DNA. *Nature (London)* **282**, 471–477.
- Porter, K. R., Claude, A., and Fullam, E. (1945). A study of tissue culture cells by electron microscopy. *J. Exp. Med.* **81**, 233–244.
- Poyton, R. O. (1983). Memory and membranes: The expression of genetic and spatial memory during the assembly of organelle macrocompartments. In "Modern Cell Biology," Vol. 2, pp. 15–72. Liss, New York.
- Rachubinski, R. A., Verma, D. P. S., and Bergeron, J. J. M. (1980). Synthesis of rat liver microsomal cytochrome b_5 by free ribosomes. *J. Cell Biol.* **84**, 705–716.
- Reitman, M., and Kornfeld, S. (1981). UDP-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosamine-1-phosphotransferase: Proposed enzyme for the phosphorylation of the high mannose oligosaccharide units of lysosomal enzymes. *J. Biol. Chem.* **256**, 4275–4281.
- Reitman, M., Varki, A., and Kornfeld, S. (1981). Fibroblasts from patients with I-cell disease and pseudo-Hurler polydistrophy are deficient in UDP-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosaminylphosphotransferase activity. *J. Clin. Invest.* **67**, 1574–1579.
- Renooij, W., van Golde, L. M. G., Zwaal, R. F. A., Roelofsens, B., and van Deenen, L. L. M. (1974). Preferential incorporation of fatty acids at the inside of human erythrocyte membranes. *Biochim. Biophys. Acta* **363**, 287–292.
- Ribgy, P. W. J. (1982). Expression of cloned genes in eukaryotic cells using vector systems derived

- from viral replicons. In "Genetic Engineering" (R. Williamson, ed.), Vol. 3, pp. 83-141. Academic Press, New York.
- Rindler, M. J., Ivanov, I. E., Rodriguez-Boulán, E., and Sabatini, D. D. (1982). Biogenesis of epithelial cell plasma membranes. *Ciba Found. Symp.* **92**, 184-202.
- Rindler, M. J., Ivanov, I. E., Plesken, H., Rodriguez-Boulán, E., and Sabatini, D. D. (1984). Viral glycoproteins destined for apical or basolateral plasma membrane domains traverse the same Golgi apparatus during their intracellular transport in doubly infected Madin-Darby Canine Kidney cells. *J. Cell Biol.* **98**, 1304-1319.
- Robbins, P. W., Hubbard, C., Turco, S. J., and Wirth, D. F. (1977). Proposal for a common oligosaccharide intermediate in the synthesis of membrane glycoproteins. *Cell* **12**, 893-900.
- Rodewald, R. (1973). Intestinal transport of antibodies in the newborn rat. *J. Cell Biol.* **58**, 189-211.
- Rodewald, R. (1980). Distribution of immunoglobulin G receptors in the small intestine of the young rat. *J. Cell Biol.* **85**, 18-32.
- Rodriguez-Boulán, E. (1983). Membrane biogenesis, enveloped RNA viruses and epithelial polarity. In "Modern Cell Biology," Vol. 1, pp. 119-170. Liss, New York.
- Rodriguez-Boulán, E., and Pendergast, M. (1980). Polarized distribution of viral envelope proteins in the plasma membrane of infected epithelial cells. *Cell* **20**, 45-54.
- Rodriguez-Boulán, E., and Sabatini, D. D. (1978). Asymmetric budding of viruses in epithelial monolayers: A model system for study of epithelial polarity. *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5071-5075.
- Rodriguez-Boulán, E., Kreibich, G., and Sabatini, D. D. (1978a). Spatial orientation of glycoproteins in membranes of rat liver rough microsomes. I. Localization of lectin-binding sites in microsomal membranes. *J. Cell Biol.* **78**, 874-893.
- Rodriguez-Boulán, E., Sabatini, D. D., Pereyra, B. N., and Kreibich, G. (1978b). Spatial orientation of glycoproteins in membranes of rat liver rough microsomes. II. Transmembrane disposition and characterization of glycoproteins. *J. Cell Biol.* **78**, 894-909.
- Rodriguez-Boulán, E., Paskiet, K. T., and Sabatini, D. D. (1983). Assembly of enveloped viruses in MDCK cells: Polarized budding from single attached cells and from clusters of cells in suspension. *J. Cell Biol.* **96**, 866-874.
- Rodriguez-Boulán, E., Paskiet, K. T., Salas, P. J. I., and Bard, E. (1984). Intracellular transport of influenza virus hemagglutinin to the apical surface of Madin-Darby Canine Kidney cells. *J. Cell Biol.* **98**, 308-319.
- Rogers, J., Early, P., Carter, C., Calame, K., Bond, M., Hood, L., and Wall, R. (1980). Two mRNAs with different 3' ends encode membrane-bound and secreted forms of immunoglobulin μ chain. *Cell* **20**, 303-312.
- Rose, J. K., and Bergman, J. E. (1982). Expression from cloned cDNA of cell-surface secreted forms of the glycoprotein of vesicular stomatitis virus in eukaryotic cells. *Cell* **30**, 753-762.
- Rose, J. K., and Bergman, J. E. (1983). Altered cytoplasmic domains affect intracellular transport of the vesicular stomatitis virus glycoprotein. *Cell* **34**, 513-524.
- Rose, J. K., Welch, W. J., Sefton, B. M., Esch, F. S., and Ling, N. C. (1980). Vesicular stomatitis virus glycoprotein is anchored in the viral membrane by a hydrophobic domain near the COOH terminus. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3884-3888.
- Rosenfeld, M. G., Kreibich, G., Popov, D., Kato, K., and Sabatini, D. D. (1982). Biosynthesis of lysosomal hydrolases: Their synthesis in bound polysomes and the role of co- and post-translational processing in determining their subcellular distribution. *J. Cell Biol.* **93**, 135-143.
- Roth, J., and Berger, E. G. (1982). Immunocytochemical localization of galactosyl transferase in HeLa cells: Codistribution with thiamine pyrophosphatase in trans golgi cisternae. *J. Cell Biol.* **92**, 223-229.
- Roth, M. G., Fitzpatrick, J., and Compans, R. W. (1979). Polarity of influenza and vesicular

- stomatitis virus maturation in MDCK cells: Lack of requirement for glycosylation of viral glycoproteins. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6430–6434.
- Roth, M. G., Compans, R. W., Giusti, L., Davis, A. R., Nayak, D. P., Gething, M. J., and Sambrook, J. (1983). Influenza virus hemagglutinin expression is polarized in cells infected with recombinant SV40 viruses carrying cloned hemagglutinin DNA. *Cell* **33**, 435–443.
- Rothman, J. E. (1981). The Golgi apparatus: Two organelles in tandem. *Science* **213**, 1212–1219.
- Rothman, J. E., and Fine, R. E. (1980). Coated vesicles transport newly synthesized membrane glycoproteins from endoplasmic reticulum to plasma membrane in two successive stages. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 780–784.
- Rothman, J. E., and Fries, E. (1981). Transport of newly synthesized vesicular stomatitis viral glycoprotein to purified Golgi membranes. *J. Cell Biol.* **89**, 162–168.
- Rothman, J. E., and Lenard, J. (1977). Membrane asymmetry. The nature of membrane asymmetry provides clues to the puzzle of how membranes are assembled. *Science* **195**, 743–753.
- Rothman, J. E., and Lodish, H. F. (1977). Synchronized transmembrane insertion and glycosylation of a nascent membrane protein. *Nature (London)* **269**, 775–780.
- Rothman, J. E., Bursztyjn-Pettegrew, H., and Fine, R. E. (1980). Transport of the membrane glycoprotein of vesicular stomatitis virus to the cell surface in two stages by clathrin coated vesicles. *J. Cell Biol.* **86**, 162–171.
- Sabatini, D. D., Kreibich, G., Morimoto, T., and Adesnik, M. (1982). Mechanisms for the incorporation of proteins in membranes and organelles. *J. Cell Biol.* **92**, 1–22.
- Salas, P. J. I., Vega-Salas, D. E., Cerejido, M., and Rodriguez-Boulan, E. (1985). Role of cytoskeleton in epithelial cell polarity. Effect of cytochalasin D and Colchicine on the polarized budding of enveloped viruses from Madin–Darby Canine Kidney (MDCK) cells. Submitted.
- Schachter, H., and Roseman, S. (1980). Mammalian glycosyl-transferases. In "The Biochemistry of Glycoproteins and Proteoglycans" (W. Lennarz, ed.), pp. 85–160. Plenum, New York.
- Schekman, R. (1982). The secretory pathway in the yeast. *Trends Biochem. Sci.* **7**, 243–246.
- Schmidt, M. F. G., and Schlesinger, M. (1979). Fatty acid binding to vesicular stomatitis virus glycoprotein: A new type of post-translational modification of the viral glycoprotein. *Cell* **17**, 813–819.
- Schneider, D. L. (1981). ATP-dependent acidification of intact and disrupted lysosomes. Evidence for an ATP-driven proton pump. *J. Biol. Chem.* **256**, 3858–3864.
- Semenza, G. (1976). Small intestinal disaccharidases: their properties and role as sugar translocators across natural and artificial membranes. In "The Enzymes of Biological Membranes" (A. Martonosi, ed.). Plenum, New York.
- Shida, H., and Matsumoto, S. (1983). Analysis of the hemagglutinin glycoprotein from mutants of vaccinia virus that accumulates on the nuclear envelope. *Cell* **33**, 423–434.
- Simons, K., and Garoff, H. (1980). The budding mechanisms of enveloped animal viruses. *J. Gen. Virol.* **50**, 1–21.
- Singer, P. A., and Williamson, A. R. (1980). Different species of messenger RNA encode receptor and secretory IgM chains differing at their carboxyl termini. *Nature (London)* **285**, 294–299.
- Sleigh, M. J., Both, G. W., Brownlee, G. G., Bender, V. J., and Moss, B. A. (1980). The haemagglutinin gene of influenza A virus: nucleotide sequence analysis of cloned DNA copies. In "Structure and variation in influenza virus" (W. G. Laver and G. M. Air, eds.), pp. 69–78. Elsevier, Amsterdam.
- Sly, W. (1982). The uptake and transport of lysosomal enzymes. In "The Glycoconjugates" (M. I. Horowitz, ed.), Vol. IV, pp. 3–25. Academic Press, New York.
- Solari, R., and Kraehenbuhl, J. P. (1984). Biosynthesis of the IgA antibody receptor: A model for the transepithelial sorting of a membrane glycoprotein. *Cell* **36**, 61–71.
- Sprague, J., Condra, J. H., Amheiter, H., and Lazzarini, R. A. (1983). Expression of a recombinant DNA gene coding for the vesicular stomatitis virus nucleocapsid protein, *J. Virol.* **45**, 773–781.

- Srinivas, R. V., Alonso-Caplen, F. V., and Compans, R. W. (1983). Glycosylation and transport of two viral membrane glycoproteins that are anchored by amino-terminal signal peptide sequences. *J. Cell Biol.* **97**, 444a.
- Steck, T. L. (1978). The band 3 protein of the human red cell membrane: A review. *J. Supramol. Struct.* **8**, 311-324.
- Steiner, D. F., Clark, J. L., Nolan, C., Rubenstein, A. H., Margoliash, E., Melani, F., and Oyer, P. E. (1970). The biosynthesis of insulin and some speculations regarding the pathogenesis of human diabetes. In "The Pathogenesis of Diabetes Mellitus" (E. Cerasi and R. Luft, eds.), pp. 123-132. Almqvist & Wiksell, Stockholm.
- Steinman, R. M., Brodie, S. E., and Cohn, Z. A. (1976). Membrane flow during pinocytosis. A stereologic analysis. *J. Cell Biol.* **68**, 665-687.
- Steinman, R. M., Mellman, I. S., Muller, W. A., and Cohn, Z. A. (1983). Endocytosis and the recycling of plasma membrane. *J. Cell Biol.* **96**, 1-27.
- Strawser, L. D., and Touster, O. (1980). The cellular processing of lysosomal enzymes and related proteins. *Rev. Physiol. Biochem. Pharmacol.* **87**, 169-210.
- Strous, G. J. A. M., and Berger, E. C. (1982). Biosynthesis, intracellular transport and release of the Golgi enzyme galactosyl transferase (lactose synthetase A protein) in HeLa cells. *J. Biol. Chem.* **257**, 7623-7628.
- Strous, G. J. A. M., and Lodish, H. F. (1980). Intracellular transport of secretory and membrane proteins in hepatoma cells infected by vesicular stomatitis virus. *Cell* **22**, 709-717.
- Strous, G. J., van Kerkhof, P., Willemsen, R., Geuze, H. J., and Berger, E. C. (1983). Transport and topology of galactosyl transferase in endomembranes of HeLa cells. *J. Cell Biol.* **97**, 723-727.
- Sturman, L. S., and Holmes, K. V. (1983). The molecular biology of corona viruses. *Adv. Virus Res.* **28**, 35-112.
- Sveda, M. M., and Lai, C. J. (1981). Functional expression in primate cells of cloned cDNA coding for the hemagglutinin surface glycoprotein of influenza virus. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5488-5492.
- Sveda, M. M., Markoff, L., and Lai, C. J. (1982). Cell surface expression of the influenza virus hemagglutinin requires the hydrophobic carboxy-terminal sequences. *Cell* **30**, 649-656.
- Tabas, I., and Kornfeld, S. (1980). Biosynthetic intermediates of beta-glucuronidase contain high mannose oligosaccharides with blocked phosphate residues. *J. Biol. Chem.* **255**, 6633-6639.
- Tabas, I., Schlesinger, S., and Kornfeld, S. (1978). Processing of high mannose oligosaccharides to form complex type oligosaccharides on the newly synthesized polypeptides of the vesicular stomatitis virus G protein and the IgG heavy chain. *J. Biol. Chem.* **253**, 716-722.
- Tartakoff, A. M. (1980). The Golgi complex: Crossroads for vesicular traffic. *Int. Rev. Exp. Pathol.* **22**, 228-251.
- Tartakoff, A. M. (1983). Perturbation of vesicular traffic with the carboxylic ionophore monensin. *Cell* **32**, 1026-1028.
- Tartakoff, A. M., and Vassalli, P. (1977). Plasma cell immunoglobulin secretion. Arrest is accompanied by alterations in the Golgi complex. *J. Exp. Med.* **146**, 1332-1345.
- Tartakoff, A. M., and Vassalli, P. (1983). Lectin binding sites as markers of Golgi subcompartments: Proximal to distal maturation of oligosaccharides. *J. Cell Biol.* **97**, 1243-1248.
- Tkacz, J. S., and Lampen, J. O. (1972). Wall replication in *Saccharomyces* species: use of fluorescein-conjugated concanavalin A to reveal the site of mannan insertion. *J. Gen. Microbiol.* **72**, 243-247.
- Tomita, M., and Marchesi, V. T. (1975). Aminoacid sequence and oligosaccharide attachment sites of human erythrocyte glycophorin. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2964-2968.

- Tycko, B., and Maxfield, F. R. (1982). Rapid acidification of endocytic vesicles containing alpha-2 macroglobulin. *Cell* **28**, 643-651.
- Van Golde, L. M. G., Raben, J., Batenburg, J. J., Fleischer, B., Zambrano, F., and Fleischer, S. (1974). Biosynthesis of lipids in Golgi complex and other subcellular fractions from rat liver. *Biochim. Biophys. Acta* **360**, 179-192.
- Varghese, J. N., Laver, W. G., and Colman, P. M. (1983). Structure of the influenza virus glycoprotein antigen neuraminidase at 2.9 Å resolution. *Nature (London)* **303**, 35-40.
- Varki, A., and Kornfeld, S. (1980). Identification of a rat liver *N*-acetylglucosaminyl phosphodiesterase capable of removing "blocking" *N*-acetylglucosamine residues from phosphorylated high mannose oligosaccharides of lysosomal enzymes. *J. Biol. Chem.* **255**, 8398-8401.
- Vasalli, P., Tedghi, R., Lisowska-Bernstein, B., Tartakoff, A., and Jaton, J.-C. (1979). Evidence for hydrophobic region within heavy chains of mouse B lymphocyte membrane-bound IgM. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5515-5519.
- Verkleij, A. J., Zwaal, R. F. A., Roelofs, B., Comfurius, P., Kastelijn, D., and van Deenen, L. L. M. (1973). The asymmetric distribution of phospholipids in the human red cell membrane. A combined study using phospholipases and freeze-etch electron microscopy. *Biochim. Biophys. Acta* **323**, 178-193.
- Von Figura, K., Rey, M., Printz, R., Voss, B., and Ullrich, K. (1979). Effect of tunicamycin on transport of lysosomal enzymes in cultured skin fibroblasts. *Eur. J. Biochem.* **101**, 103-109.
- Wall, D. A., Wilson, G., and Hubbard, A. L. (1980). The galactose-specific recognition system of mammalian liver: The route of ligand internalization in rat hepatocytes. *Cell* **21**, 79-93.
- Walter, P., and Blobel, G. (1980). Purification of a membrane associated protein complex required for protein translocation across the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7112-7116.
- Walter, P., and Blobel, G. (1981a). 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 protein. *J. Cell Biol.* **91**, 551-556.
- Walter, P., and Blobel, G. (1981b). Translocation of proteins across the endoplasmic reticulum. III. Signal recognition protein (SRP) causes signal sequence dependent and site specific arrest of chain elongation that is released by microsomal membranes. *J. Cell Biol.* **91**, 557-561.
- Walter, P., and Blobel, G. (1982). Signal recognition particle contains a 7s RNA essential for protein translocation across the endoplasmic reticulum. *Nature (London)* **299**, 691-698.
- Walter, P., Ibrahimi, I., and 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**, 545-550.
- Ward, C. W., and Dopheide, T. A. (1979). Primary structure of the Hong Kong (H3) hemagglutinin. *Br. Med. Bull.* **35**, 51-56.
- Waterfield, M. D., Espelie, K., Elder, K., and Skehel, J. J. (1979). Structure of the hemagglutinin of influenza virus. *Br. Med. Bull.* **35**, 57-73.
- Wehland, J., Willingham, M. C., Gallo, M. G., and Pastan, I. (1982). The morphologic pathway of exocytosis of the vesicular stomatitis virus G protein in cultured fibroblasts. *Cell* **28**, 831-841.
- Whaley, W. G., and Dauwalder, M. (1979). The Golgi apparatus, the plasma membrane and functional integration. *Int. Rev. Cytol.* **58**, 199-245.
- White, J., Keelien, M., and Helenius, A. (1983). Membrane fusion proteins of enveloped viruses. *Q. Rev. Biophys.* **16**, 151-195.
- Wilson, I. A., Skehel, J. J., and Wiley, D. C. (1981). Structure of the hemagglutinin membrane glycoprotein of influenza virus at 3 Å resolution. *Nature (London)* **289**, 366-373.
- Winter, G., Fields, S., and Brownlee, G. G. (1981). Nucleotide sequence of the hemagglutinin gene of a human influenza virus H1 subtype. *Nature (London)* **292**, 72-75.

- Yost, S. C., Hedgpeth, J., and Lingappa, V. R. (1983). A stop transfer sequence confers predictable orientation to a previously secreted protein in cell free systems. *Cell* **34**, 759-766.
- Young, R. W. (1973). The role of Golgi complex in sulfate metabolism. *J. Cell Biol.* **57**, 175-189.
- Ziomek, C. A., Schulman, S., and Edidin, M. (1980). Redistribution of membrane proteins in isolated mouse intestinal cells. *J. Cell Biol.* **86**, 849-857.
- Zuniga, M. C., Malissen, B., McMillan, M., Brayton, P. R., Clark, S. S., Forman, J., and Hood, L. (1983). Expression and function of transplantation antigens with altered or deleted cytoplasmic domains. *Cell* **34**, 535-544.