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Traffic of Proteins and Compartmentalization of Eukaryotic Cells

The Organism Is a Life Cycle

A multicellular living being begins as a single cell. It grows in size by cell division and divides the labor among the newly formed cells by differentiation. An adult human being has about 10^{14} cells. These are organized into different organs, such as the heart, the brain, and the digestive tract, each made of different subpopulations of cells forming tissues. The different cells in each tissue have the same complement of genes as the fertilized egg from which they derive. However, only a part of the genetic program is expressed in each tissue cell. The set of proteins that is produced in the cells of the stomach is different from those of the neurons in the brain. These proteins give each cell its functional properties. For instance, the cells lining the stomach secrete into the gastric juice both hydrochloric acid and the digestive enzyme pepsin, a protein. At the same time these cells form a tight layer lining the stomach wall which is impermeable to this acid juice of pepsin, which, if it could penetrate between the cells into the "milieu interieur," would have catastrophic consequences. The perforation of a gastric ulcer is an example of what happens when this lining fails. The nerve cells in the brain, on the other hand, form long processes called axons and dendrites which connect with other nerve cells. The axon of one neuron contacts the dendrite of another neuron and these contact sites, which are responsible for the transmission of the information, are called synapses. To assemble the synaptic networks in the brain each neuron has to deliver one set of proteins to the axon and another to the dendrites.

Currently, very little is known about the mechanisms responsible for execution of the developmental program in any organism. To investigate this problem we must define the complex sequence of events which lead to the commitment of each cell type to a specific genetic program. However, differentiation is not understood simply by defining how genes are turned on and off during embryonic development. We also have to elucidate the mechanisms by which cells execute the genetic program and organize themselves to fulfill their functions.

Nerve cells and gastric cells look completely different under the microscope. The cells have a characteristic size, form, and architecture. Moreover, cells interact in a precise way with their neighboring cells to form a tissue which has a specific size and form. We have already mentioned the case of neurons that form synapses, but many other cell types in the body communicate by different modes, leading to a remarkable array of intercellular interactions. These are three-dimensional problems with the additional fourth dimension of time included to effect change. We are starting to get glimpses of the mechanisms that cells employ to organize themselves during embryonic development, but we are still far from understanding how cells accomplish their complex tasks.

The Cell Is Compartmentalized

An animal cell is like a city suspended under water and surrounded by a flexible shell. More than 90% of every vertebrate is water. Life on Earth in all likelihood has its origins in the primeval sea. Every living organism on this planet is probably the descendant of a single cell dividing in the primeval soup more than 3 billion years ago as the earth was cooling. Without water, life in its present form would be inconceivable. The outer shell of the cell is called the plasma membrane. This membrane is not totally impermeable but regulates import and export activities of the cell. Inside the cell there are several compartments with different functions. The most obvious, because of its size, is the nucleus (Fig. 1). The nucleus is a repository of the cell's genetic library, the information being encoded in a four-letter code in the nucleic acid molecules organized into chromosomes. In the nucleus the decisions are made as to which set of genes is to be transcribed and then translated into specific proteins. These decisions are not made without external influence. Gene expression is regulated by chemical signals which reach the nucleus from the plasma membrane, which is in continuous contact with the surrounding environment and with neighboring cells. Hormones and growth factors circulating in the body fluids bind to specific proteins in the plasma membrane. The binding stimulates the release of chemical messengers inside the cell which relay signals across the cytosol to the nucleus.

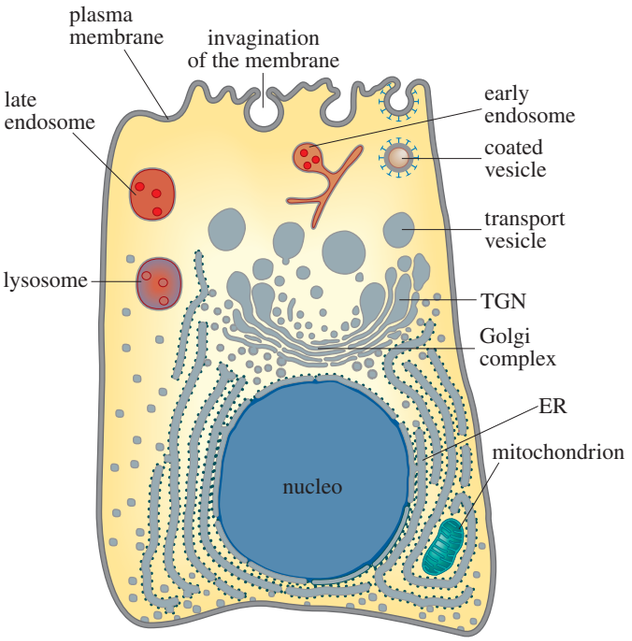

FIGURE 1 The principal compartments of the cell involved in the transport pathway. The nucleus is surrounded by the nuclear envelope which is continuous with the endoplasmic reticulum (ER), the site of synthesis of secretory proteins. These proteins and lipids pass through the Golgi complex, successively reaching the trans-Golgi network (TGN), from which transport vesicles target them to the plasma membrane. The caveolae and the coated vesicles are involved in the opposite process from the membrane to the cell interior. Early and late endosomes and lysosomes play different roles in the sorting and degradation of proteins, lipids, and solutes internalized in the cell or coming from the biosynthetic pathway.

Another conspicuous compartment is the mitochondrion. The mitochondria are the power plants of the cell which use oxygen to burn foodstuffs providing the cell with chemical energy. The endoplasmic reticulum (ER) forms a large network of membranes that produce proteins for export from the cell as well as for other intracellular destinations. These proteins have to pass the Golgi complex, which is a postal office for the distribution of cellular components to their addresses. Among these are the lysosomes, which are garbage dumps with built-in recycling capacity.

All these cellular compartments have specific sizes, shapes, and locations within the cell depending on the cell type in which they are harbored. They are all bounded by membranes which are similar in structure to that of the plasma membrane. The membranes consist of two closely apposed layers of lipids and they form a framework as flexible as soap bubbles but stable in structure. Most of the membrane lipids, such as phospholipids and cholesterol, are synthesized in the ER. From there, these lipids are, like

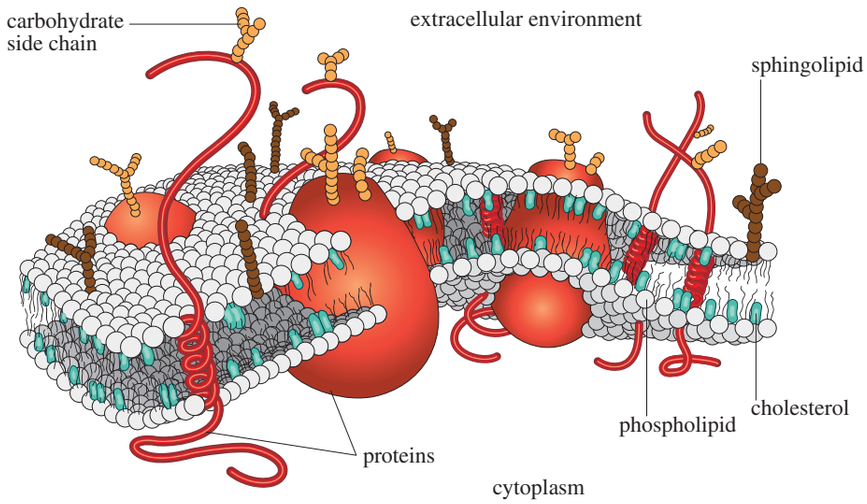

FIGURE 2 The membrane is formed by two juxtaposed leaflets of lipids which, together with the proteins that float there, contribute to the asymmetry of this fluid but stable structure. Phospholipids and cholesterol are present on both sides of the lipid bilayer, whereas sphingolipids are located only on the outer leaflet. Some proteins also have distinct topological orientations: For example, the carbohydrate side chains of the glycoproteins face the outside. The membranes of the different cell compartments have a similar basic organization, but they vary in their lipid and protein composition.

proteins, transported to other membrane-bound compartments. Another lipid class is the sphingolipids. These are produced in the Golgi complex. The lipid bilayer in each membrane behaves like a two-dimensional liquid in which the membrane proteins swim around (Fig. 2). As discussed later, the bilayer is not a homogeneous mixture of its lipid constituents. They are asymmetrically disposed over the outer and inner leaflets of the bilayer. Also, the proteins have a precise topology which is generated during synthesis. Parts of the proteins are facing the lumen of the compartment and others are exposed to the interior of the cell. It is important to note that each cellular compartment has its own characteristic set of proteins which carry out functions specific for each compartment. The internal fluid inside the cell into which these cellular compartments are suspended is not water but rather a formless jelly called the cytosol. It is also bolstered by numerous cables crossing the cytosol in different and often carefully specified directions. These cables form the cytoskeleton, and some of these bridge different compartments to each other. The cytoskeleton is made of three different types of filaments that have different diameters and play different roles: Actin filaments and microtubules are the best characterized and are the thinnest and largest, respectively. In addition, there are intermediate filaments whose function is less clear. Unlike what is generally assumed, these cables are not static but extremely dynamic. Actin filaments and microtubules undergo continuous remodeling to serve the needs of the cell in helping to generate the exterior and architecture of cells. Microtubules play a pivotal role in cell division when they disappear from the cell periphery and polymerize to form the mitotic spindle, which is the cellular apparatus that controls the segregation of the chromosomes to the two daughter cells. Microtubules also constitute tracks which are used to transport cargo across long distances, for example, from the basal to the apical pole in epithelial cells or along the axon in neurons. Actin filaments are well-known to function in muscle contraction. They are also important for cell motility, cell shape, and vesicular transport.

Proteins Must Find Their Compartment in the Cell

Cells are continuously renewing their constituents, like the servicing of modern aircraft: The parts are checked and replaced at specified intervals. This is the case with all the proteins which a cell contains. The cellular proteins are synthesized in the cytosol. The only exceptions are proteins belonging to the mitochondrion. Mitochondria are special because they are probably derived from primitive bacteria which swam into the ancestral precursors of animal cells and remained there to specialize in power plants. They retained some of their genes and also the machinery for transcribing genes and synthesizing proteins. This means that some mitochondrial proteins are specified by nuclear genes and some by the mitochondrial genes, and these latter ones

are transcribed and translated inside the mitochondrion into proteins.

Newly synthesized proteins in the cytosol have to find the site in the cell where they belong. This poses no problem for the proteins which perform their function in the cytosol but concerns all the others destined to other locations in the cell. This sorting puzzle which comprises thousands of different proteins in each cell is being studied in laboratories throughout the world. The solutions to this problem lie at the heart of cellular organization. The first clue came from studies by Cesar Milstein in Cambridge and the problem was elucidated in New York by Günther Blobel and Bernhard Dobberstein (Blobel and Dobberstein, 1975; Walter and Lingappa, 1986). They found that proteins secreted by cells have a small extra segment which functions as a postal address for the ER into which these proteins are delivered from the cytosol. The same postal code is used not only by proteins belonging to the ER but also by proteins destined for the Golgi complex, the lysosomes, the plasma membrane, and export from the cells. Other studies have revealed additional categories of postal addresses which are used for transport of mitochondrial and of nuclear proteins from the cytosol to their respective destinations. These three different classes of postal tags are usually not only necessary but also sufficient. When they are tagged artificially onto a bona fide cytosolic protein, the hybrid protein is routed to the ER, the mitochondria, or the nucleus, depending on the tag that was used. Such experiments are routinely performed by splicing the gene segment coding for the postal tag to a gene coding for the cytosolic protein and then expressing this hybrid gene in the test tube or in a suitable animal cell. Usually, the postal addresses can be read correctly by cells from many different species.

The cellular mechanisms for reading the postal codes and routing the proteins to different addresses in the cell have only partially been elucidated. In the case of proteins addressed to the ER, it is known that there is a an escort system to guarantee that proteins that have to be secreted cannot accumulate in the cytosol. This is achieved by a multiprotein complex assembled around a RNA backbone called the signal recognition particle in the cytosol which binds to the postal tag on the protein while it is still being synthesized (Fig. 3). This complex docks to another protein protruding from the ER called the signal recognition par-

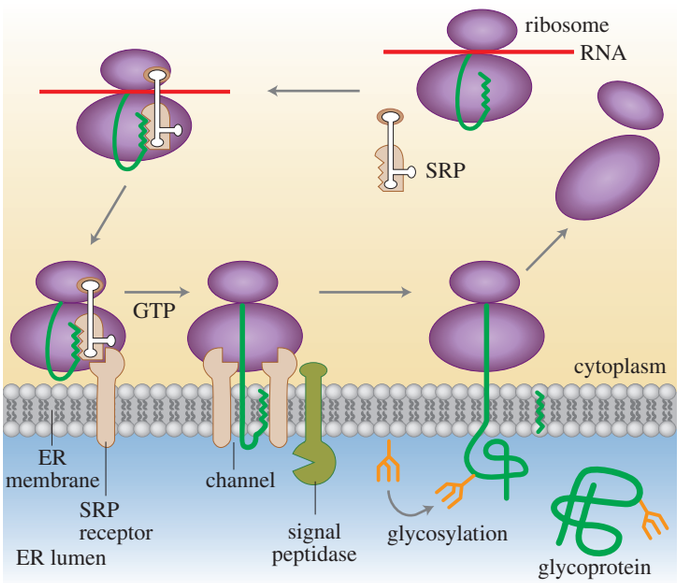

FIGURE 3 Schematic representation of translocation of a protein across the membrane of the endoplasmic reticulum. A secretory protein (green chain) is translated from the corresponding mRNA by a ribosome; as synthesis proceeds a hydrophobic fragment (signal peptide, wavy line) of the protein protrudes from the ribosome. Successively, a ribonucleoprotein complex SRP binds to this signal peptide in the cytosol. At this point protein translation is arrested to allow the ribosome–protein–SRP complex to dock to the SRP receptor present on the ER membrane. Protein translation is resumed in a reaction that requires GTP and the opening of a protein channel through which translocation across the membrane of the ER takes place. A specific protease, signal peptidase, cleaves the signal peptide off the protein. Specialized enzymes then catalyze the addition of carbohydrate side chains to the protein (glycosylation) which then becomes a glycoprotein. After translocation is complete the ribosome is released into the cytosol ready to bind another mRNA molecule to initiate a new cycle of protein synthesis.

ticle receptor. This docking event initiates the passage of the protein being synthesized into the ER. This translocation across the membrane takes place through a protein pore in the bilayer and is often accompanied by the addition of carbohydrate side chains to the protein, which then becomes a glycoprotein. Similar recognition devices and docking proteins exist for mitochondria and nuclei.

The sorting puzzle is further complicated by the fact that proteins routed to one membrane-bound compartment have destinations beyond the first membrane they encounter. The mitochondrion consists of two membranes, each with a specific set of proteins. Also, the nucleus has two membranes. The problem is even more complicated for those proteins that are routed from the ER to the Golgi complex, the lysosomes, or the plasma membrane. These proteins make use of membrane-bound containers called vesicles which bud off from one cellular compartment like soap bubbles to be fused with the next. In fact, the cytosol is full of these membrane bubbles which move about inside the cell.

Transport vesicles allow proteins that follow the biosynthetic pathway (Fig. 4) to be transported from the ER to the Golgi complex and from its exit site, the trans-Golgi network, to the plasma membrane (Rothman and Orci, 1992). From the plasma membrane vesicles containing fluid lipids and proteins are continuously internalized (Gruenberg and Maxfield, 1995). Clathrin-coated vesicles depart and transport their cargo to the first station of the endocytic pathway (Fig. 5), the early endosome. Depending on their function, some molecules can be returned from here to the plasma membrane either directly or by passing through a recycling endosome. Molecules which are destined for degradation continue their journey into compartments which are progressively more acidic and richer in hydrolases, late endosomes, and lysosomes.

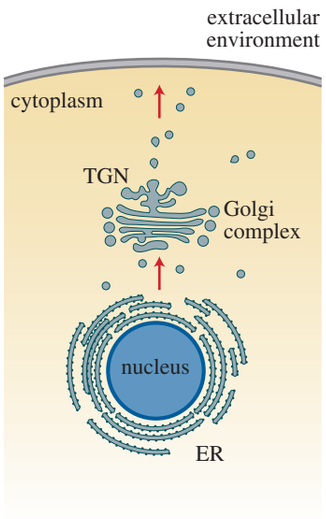

FIGURE 4 The biosynthetic pathway. Proteins destined to the biosynthetic pathway are first translocated across the endoplasmic reticulum (ER) membrane, packed into transport vesicles, and delivered to the Golgi apparatus, in which they undergo various posttranslational modifications. The exit site from the Golgi complex is through the trans-Golgi network (TGN). Here, other vesicles are formed that deliver their cargo of proteins to the plasma membrane as well as vesicles carrying lysosomal enzymes to the endosomes.

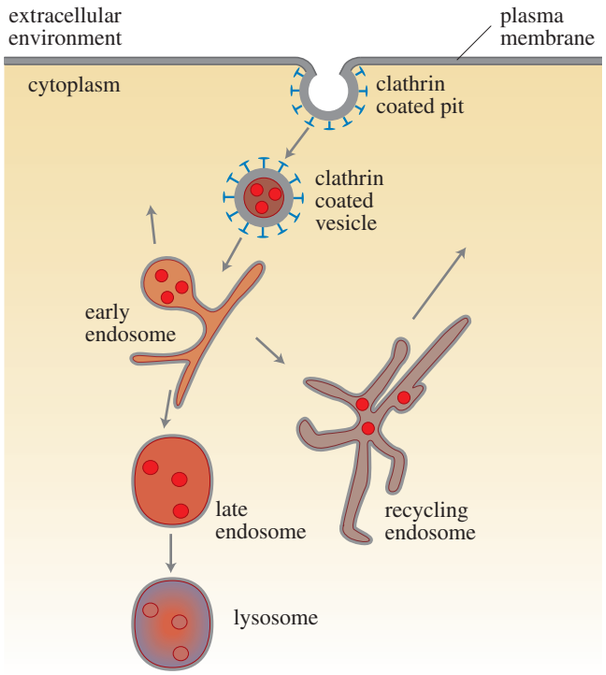

FIGURE 5 The endocytic pathway via transport vesicles. The cytosolic protein clathrin and a set of adaptors assemble on the inner surface of the plasma membrane causing the invagination of the lipid bilayer (clathrin-coated pit). Subsequently, this area of the plasma membrane pinches off in the form of a sealed membrane container (clathrin-coated vesicle) which carries membrane proteins, lipids, and solutes from the surface. These vesicles deliver their contents to the early endosomes. The internalized molecules can return to the plasma membrane either directly or by passing through the recycling endosome. Other molecules are transported into the late endosomes or into the lysosomes where they are degraded.

Viruses as Blood Bounds

Making sense out of this hubble of bubbles has taken many years. The groundwork was laid by George Palade (1975), who studied the export of proteins from cells. Further insight into how these traffic routes connect the ER with the plasma membrane has been obtained by employing mem-

brane viruses as guides inside the cells. They act as bloodhounds to follow the trail of membrane bubbles connecting intracellular compartments.

These viruses consist of a package of RNA genes which contain the instructions necessary for producing virus progeny in the host cell. This package is wrapped in a membrane similar to the plasma membrane. In fact, the virus steals a segment of the host plasma membrane when a newly formed virus particle leaves the cell. The external surface is studied with spikes giving the virus particle the appearance of a sea urchin but with dimensions so small that it can only be seen in the electron microscope.

The viruses start their tour through the host cell at the cell surface (Simons *et al.,* 1982). By following an RNA virus through the cell, we get a snapshot of how the membrane trafficking routes are organized in eukaryotic cells. The life cycle begins with the penetration of the virus into the cell. A prerequisite for this interaction to occur is that the virus recognizes chemical structures on the plasma membrane to which the virus particle can attach. Only cell types with the appropriate receptors for the virus can be infected by the virus. Soon after this attachment has occurred, the virus particle is engulfed into an invagination which forms a membrane bubble with a virus inside. The virus is now on its way into the cell interior (Fig. 6). The bubble has a coating of protein organized in a beautifully ordered lattice which plays an important role in its formation. The protein component of this clustering lattice was first discovered by Barbara Pearse (1980). The clustering coat is short-lived and is thrown off before the bubble containing the virus bumps into an intracellular compartment called the endosome and fuses with its membrane. The fusion event is like a small soap bubble fusing with a large one and melting together to form a larger bubble. After the

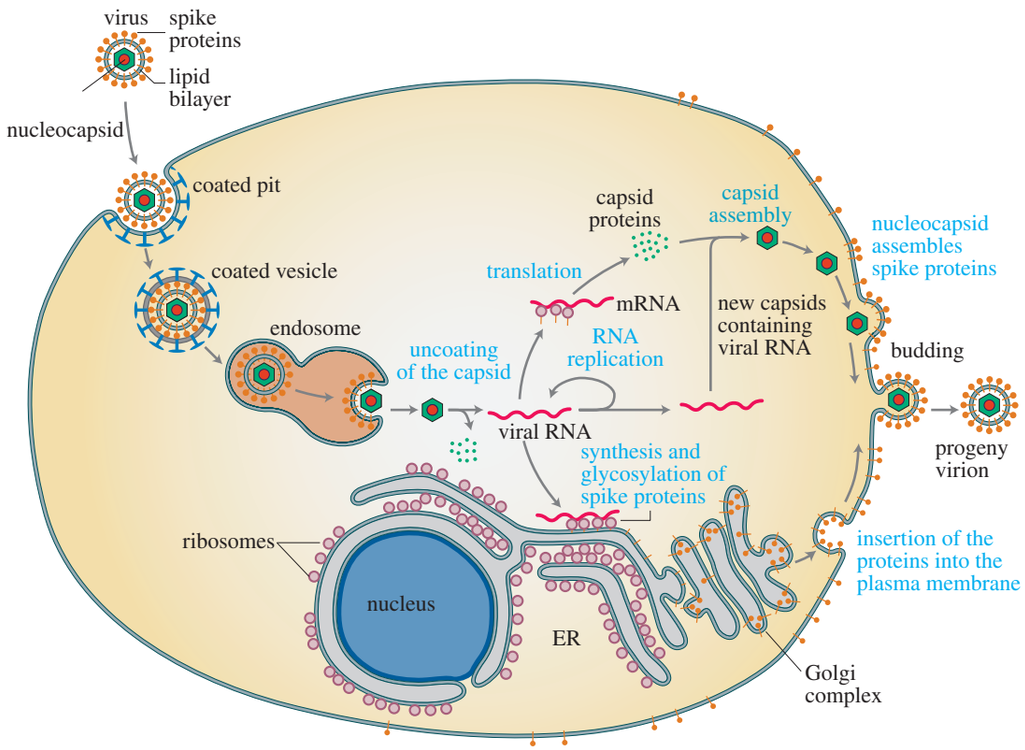

FIGURE 6 Life cycle of a virus. The virus enters the cell through coated pits and coated vesicles and reaches the endosomes, where the viral membrane fuses with that of the endosome. In this way the proteins that constitute the viral envelope are lost but the nucleocapsid, containing the viral nucleic acid, is released into the cytosol. With the uncoating of the capsid, the viral RNA is exposed and the cell unwittingly becomes a factory for virus production. Large amounts of the proteins that constitute the viral spikes are synthesized and glycosylated in the endoplasmic reticulum and transported successively to the Golgi complex (where their glycosylation is completed) and hence to the plasma membrane. In the cytosol the viral RNA is replicated into thousands of copies and is packaged again into new capsids. All viral components are now ready to be assembled into new virions. The virus gene packet can recruit the spike proteins on the plasma membrane and a newly formed virion can bud outside the cell ready to spread the infection (modified from Alberts *et al.,* 1989).

two cellular bubbles have fused with each other the virus is dumped into the endosomal interior. This causes a shock to the virus because the inside fluid of the endosome is acidic. This acid bath triggers a second fusion reaction. This time the virus is the small soap bubble which melts from the inside with a large endosome bubble. The first fusion event delivers the virus into the endosome and the second releases the virus gene package from the virus membrane into the cytosol.

This process is amazingly clever, but somehow it does not make sense. Why would the cell have such a handy mechanism to facilitate virus entry? It seems suicidal. Unfortunately for the cell, the virus relies on a Trojan horse type of deception. The virus makes use of a mechanism of engulfment which every animal cell employs normally to remove hormones and growth factors from the cell surface after they have initiated their action on cellular metabolism and growth. Also, nutrients such as iron and cholesterol are taken up into the cell by this route. There is a continuous stream of protein-coated membrane vesicles from the plasma membrane into the endosomes. The extent of this traffic is so great that the entire plasma membrane surface would be consumed in the formation of these bubbles in an hour or two depending on the cell if it were not for a recycling mechanism. On the other hand, the plasma membrane would grow too much if portions of membrane were not constantly removed. In contrast, the plasma membrane surface area is kept constant by a compensatory traffic of membrane vesicles from the endosomes. This continuous stream of vesicles in the opposite directions can be likened to a pair of escalators. The virus deceives the cell by sneaking onto the escalator into the cell which is normally reserved for other passengers. Most of the normal passengers are routed further than the endosomes; they continue on another escalator to the lysosomes, where they are destroyed as waste. For instance, the cholesterol in the blood circulation is carried by a lipoprotein that binds to receptor proteins on the cell surface which are subsequently internalized using the escalator to the endosomes. In the endosomal acid bath the lipoprotein is released from its receptor, which returns on the escalator to the cell surface to function in another round. The lipoprotein is routed to lysosomes where it is degraded and the cholesterol is released for use in the cell. Michael Brown and Joseph Goldstein found patients with genetic defects in the internalization route (Brown *et al.,* 1983). The lipoprotein in these patients can bind to its receptor on the cell surface but the complex cannot enter the cells because the receptor is defective and therefore cannot board on the escalator to the endosomes. This entry defect leads to the accumulation of cholesterol in the circulation and premature death through atherosclerosis.

After entering the endosomes, the viruses escape the stairway to death in the lysosomes by rapidly fusing with the endosome and releasing their contents into the cytosol. They leave their "coats" in the endosomal membrane, but the gene package is now in the cytosol. If an efficient way to stop the virus membrane from fusing in the acid bath was known, it would constitute a route to chemotherapy of membrane virus infection. However, this has to be done without impeding the traffic of normal passengers to the endosomes and the lysosomes, and to date this has not been possible.

The virus gene packet in the cytosol causes a grim change in cellular metabolism. The cell is reprogrammed by the virus genes to produce virus progeny. These genes are truly ruthlessly selfish. New gene packets are synthesized by the thousands, as are the spikes of the virus membrane. These spikes are made of proteins which belong to the class routed to the ER. They are translated with a normal postal tag for this destination. From the ER the spike proteins are further transported over the Golgi complex to the plasma membrane. These virus proteins follow exactly the same route to the cell surface as normal plasma membrane proteins. For instance, transplantation antigens and hormone receptors have been shown to follow the trail tracked down by the virus spike proteins. However, the virus spike proteins are produced in large numbers and they are easier for the investigator to trace in the cell than the normal multitude of different plasma membrane proteins. They have therefore been used extensively to study the pathway and the mechanism of transport to the cell surface.

The final stage in the life cycle of a membrane virus takes place at the plasma membrane of the infected cell. Here, the assembly of the virus particle is completed. Once again, the formation of a membrane bubble occurs, only this time it forms not in the cytosol but in the external world outside the cell. A virus-infected cell looks like an enormous soap bubble from which a continuous stream of small bubbles are pinching off, each with one packet of virus gene inside. This is a beautiful spectacle from a mechanistic point of view, but as a result the cell dies. However, the virus-infected cell has taught cell biologists many important lessons.

Moving Proteins from One Compartment to Another

Little is known of the mechanisms by which proteins are selectively transported from one site to another in the pathway of intracellular traffic which starts from the ER. The major sorting function is attributed to the Golgi complex. Here, proteins destined for the lysosomes, for export from the cell, and for the plasma membrane are sorted into separate membrane containers which deliver them to their correct addresses. One postal code in this pathway that has been deciphered is that involved in lysosomal routing (Kornfeld and Mellman, 1989). Enzymes active in waste

disposal and recycling in the lysosomes receive a second address tag in the Golgi complex as was discovered by Kurt von Figura and by William Sly and Stuart Kornfeld. This stamp is on the carbohydrate side chains linked to lysosomal enzymes and consists of a phosphorylation reaction of mannose residues attached to the glycoprotein. The lysosomal enzymes are subsequently bound to receptor proteins in the Golgi complex before they are packed into the membrane container which will take them to the lysosomes.

The mechanism by which a bubble, a membrane transport vesicle, forms in one compartment and is moved to the next compartment is under intensive investigation. The process can be divided into five stages: (i) the inclusion of cargo proteins into the membrane patch that forms the vesicle, (ii) the formation and the release of the vesicle from the donor compartment, (iii) the movement of the vesicle to the target compartment, (iv) the docking of the vesicle, and (v) the fusion of the vesicle with the membrane of the target compartment followed by the release of cargo (cf. the process of virus entry). The inclusion of cargo consisting of membrane proteins is often guided by signals present in the domains of the proteins exposed to the cytosol. These bind to adaptor proteins, which in turn assemble a protein scaffold, such as the clathrin coat, that bends the membrane to form a vesicle. Release of the vesicle is dependent on specific proteins that form rings around the neck of the budding vesicle and squeeze the vesicle out from the donor compartment. The lipid bilayers in the vesicle and in the donor membrane have to reseal immediately. Also, this resealing is probably facilitated by specific unidentified proteins.

After the transport vesicle has been released, it must find the correct acceptor compartment. In many cases, this movement occurs along cables formed by the cytoskeleton, usually microtubules. The transport vesicles cling like cable cars to the microtubule cables and carry "motors" which propel them along the cable to the correct destination. After arrival at the target compartment specific docking of the vesicle has to be ensured. Proteins called SNAREs, identified by James Rothman, form specific couples: one in the vesicle called a v-SNARE and another on the target membrane called a t-SNARE (Fig. 7; Rothman, 1994). After docking has occurred through the binding of a v-SNARE to a t-SNARE, fusion of the membrane of the transport vesicle with the target membrane is triggered. It is assumed that the SNAREs mediate this process as well; in addition, however, a switch mechanism must exist to control the formation of the v–tSNARE pair (Fig. 7) and its subsequent disassembly after the vesicle delivery process has been completed. Indeed, in vesicular transport, as in many other biological processes, there are chemical switches, called Rab proteins, which hydrolyze the trinucleotide guanosine triphosphate to diphosphate. This hydrolysis event causes a conformational change on the molecule which can be

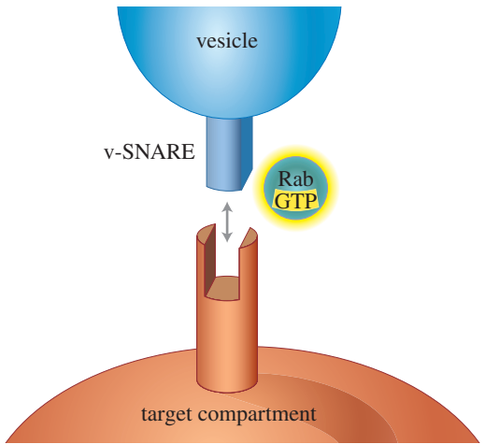

FIGURE 7 Model based on the SNARE hypothesis. Each vesicle contains a protein called vesicle SNARE (v-SNARE) which is specifically recognized by another protein located on the target compartment, t-SNARE. Only if the correct v-SNARE and t-SNARE match like a key and its lock can the vesicle dock and fuse with its acceptor compartment. This interaction, however, is regulated by a molecular switch, called Rab protein, which has to be in the active form, bound to GTP, for SNARE pairing to occur.

viewed as an on/off switch that signals to the transport machinery where and when to release the cargo proteins from the fused vesicular container (Zerial and Stenmark, 1993). Although the molecular principle is the same, every vesicular route utilizes its specific switch. Many Rab proteins (about 30) are expressed in a typical mammalian cells. These proteins are localized very specifically to distinct stations along the biosynthetic and endocytic pathways where they control trafficking from one site to another. What happens if one interferes with their switch function? This can be done by introducing mutations that alter the nucleotide binding or the hydrolysis so that the switch is blocked in the "on" or "off" position. In the case of Rab5, a Rab protein that regulates the transport from the plasma membrane to the early endosomes (Fig. 8) when the switch is blocked in the off position, endocytosis is reduced and the endosomes break down into small fragments. When the switch is blocked in the on position, then endocytosis is stimulated and the endosomes become large vacuoles. In addition to the switch function, Rab proteins also function as timers because they can remain active only for a certain period of time. This mechanism ensures that the proteins stay active for a period of time long enough to allow vesicle transport but not too long to have exaggerated organelle expansion, as in the case of the "on" mutant.

The fascinating conclusion from studies on the complex regulation of the traffic system for intracellular transport from the ER over the Golgi complex to the lysosomes and

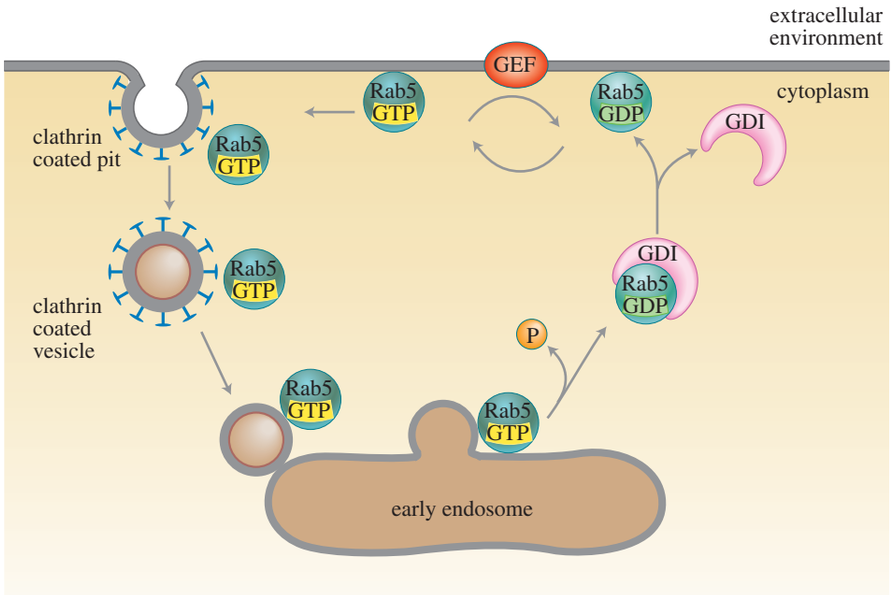

FIGURE 8 Vesicular transport regulated by the protein Rab5, one of a family of molecular switches called Rab GTPases which regulate transport from the plasma membrane to the early endosome. This regulation is controlled by the passage between the active form of Rab5, bound to GTP, and the inactive form bound to GDP. Clathrin-coated pits become clathrin-coated vesicles, which after uncoating fuse with the early endosome. For this to occur, Rab5 must be on the vesicle in the active form. Due to the GTPase activity of Rab5, GTP is hydrolyzed to GDP and Rab5 binds to GDI, an inhibitor of GDP dissociation. GDI transports the protein to the membrane, where a guanine nucleotide exchange factor, GEF (in red) converts Rab 5 to the active state, ready to initiate another cycle of vesicular transport.

the cell surface or beyond is that similar machinery is used at each vesicular transport step. Specificity is ensured by each vesicle and each target membrane having specific Rab proteins and SNAREs.

Important insights into the functioning of the Rab/SNARE machinery have come from studies of yeast cells, which are popular to molecular cell biologists because these simple unicellular organisms provide powerful genetical means to manipulate cell function. One can use yeast cells and obtain results which apply to mammalian cells because all eukaryotic cells are built on the same principles. Once a molecular solution to a central mechanism of life was "invented" during the course of evolution, it was fixed, improved, and reiterated.

Not All Cells Are the Same

It is obvious that yeast cells do not contain the solution to all problems of cellular organization. One question that has received little attention is how membrane trafficking is modulated to serve the requirements of cellular organization in different cell types. All cells have to transport newly synthesized proteins and lipids from the ER over the Golgi complex to the cell surface and to the endosome–lysosome compartments. They also have to endocytose cell surface components into endosomes and lysosomes. However, when one considers the needs of cells such as those lining the digestive tract or of neurons in the brain, radical changes in the traffic routes have to be introduced, especially in the pathways leading from the Golgi complex to the plasma membrane. Epithelial cells line the digestive tract. This lining consists of a single layer of cells connected to each other by cellular junctions. Each epithelial cell has a cell surface divided into two territories (Fig. 9): the exterior apical surface facing the lumen of the gut and the basolateral domain which receives nutrients from the blood supply. The specific traffic problem that the epithelial cells have to solve is how to provide both the apical and the basolateral plasma membrane domains with different proteins and lipids and how to organize the endocytosis routes from the two sides of the cell layer (Simons and Fuller, 1985).

The solution to this problem is novel because only the basolateral route from the Golgi complex employs the SNARE/Rab mechanism, whereas the apical pathway is of a new type. The details of this latter delivery mechanism have not been worked out. However, a central element is the involvement of lipids in the apical sorting process (Simons

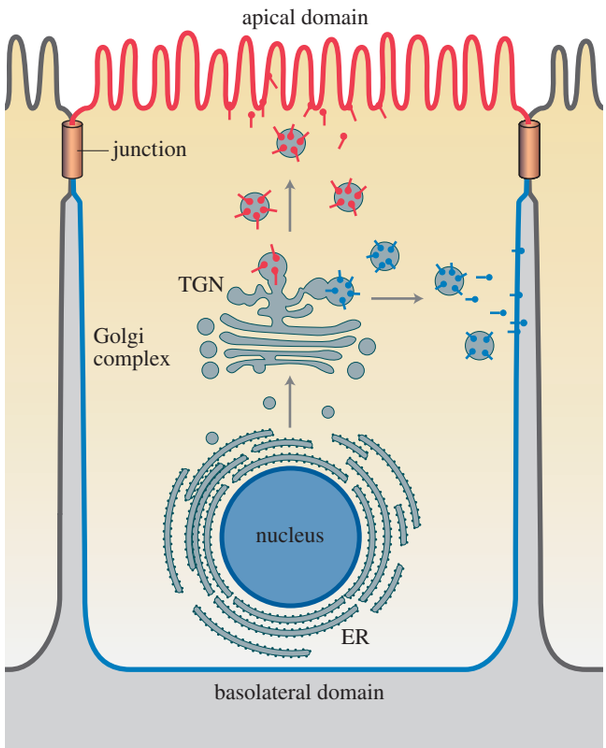

FIGURE 9 The biosynthetic pathway in a typical polarized epithelial cell. The cell surface is divided into two domains: the apical surface faces the lumen of the gut and the basolateral surface faces the bloodstream. The two plasma membrane domains have different protein and lipid contents and intermixing is prevented by the junctions which connect the cells. In the biosynthetic pathway, proteins destined for the apical or basolateral surface share a common route of transport from the ER to the Golgi complex until they reach the TGN. Here, the two sets of proteins, which have different destinations (red and blue), are sorted into distinct vesicles to be targeted to the apical (red) or basolateral (blue) plasma membrane domain.

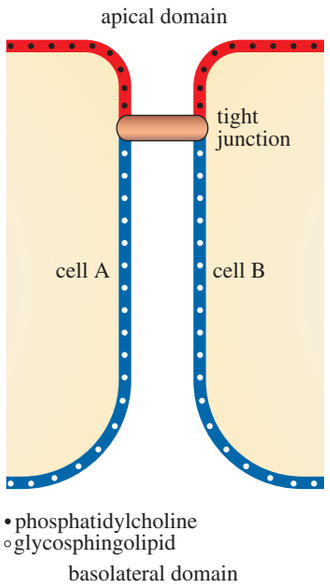

FIGURE 10 Asymmetry of the plasma membrane of polarized epithelial cells. The first level of asymmetry is determined by the fact that the apical membrane (in red) is highly enriched in sphingolipids, especially glycosphingolipids, whereas the basolateral membrane (in blue) has a higher content of phospholipids, mainly phosphatidylcholine. These cannot mix due to the presence of the tight junction. The second level of asymmetry (not shown) is determined by the fact that the glycosphingolipids and phosphatidylcholine are localized exclusively in the outer leaflet of the plasma membrane.

and Ikonen, 1997). We must again consider the organization of the lipid bilayer to understand the principles of the apical mode of membrane transport. The apical membrane (Fig. 10) is highly enriched in sphingolipids, mainly glycosphingolipids (lipids with oligosaccharides as polar headgroups), whereas the basolateral membrane contains much more phospholipids than the apical membrane, mainly phosphatidylcholine. These lipid species are localized to the outer leaflet of the plasma membrane. The tight junctions linking the epithelial cells to each other in the cell layer also form a fence that prevents mixing of the lipids and the protein, but only in the external leaflets of the apical and the basolateral lipid bilayers. The inner leaflet phospholipids (facing the cytosol) freely communicate by lateral diffusion. This segregation of sphingolipids and phosphatidylcholine takes place in the membrane of the trans-Golgi network followed by segregated delivery to the correct surface domain. Mechanistically, segregation in the external leaflet of the membrane of the Golgi complex is achieved by a clustering of the glycosphingolipids with each other. The association is facilitated by cholesterol molecules intercalating between the hydrocarbon chains of the sphingolipid molecules. These sphingolipid–cholesterol clusters behave like rafts in the fluid bilayer (Fig. 11). The most interesting property of these lipid rafts is that they specifically associate with proteins that are routed to the apical membrane in epithelial cells. Apical proteins have specific affinity for raft lipids. Thus, the sphingolipid–cholesterol rafts function as platforms for delivery of specific protein cargo to the apical membrane.

This novel mode of membrane trafficking is not restricted to epithelial cells; neurons also employ this mode of delivery for proteins directed to the axon (Fig. 12; Dotti and Simons, 1990). Surprisingly, recent studies have demonstrated that nonpolarized cells such as fibroblasts, which are present in connected tissue, have apical and basolateral cognate routes from the Golgi complex to the cell surface

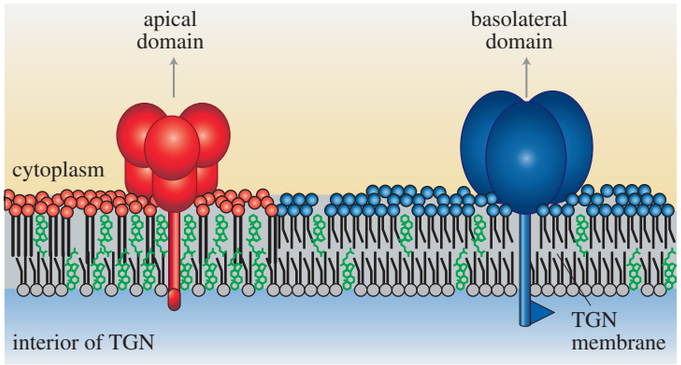

FIGURE 11 The raft model and the microdomain organization of the lipid bilayer. In the TGN, apically directed proteins (in red) are sorted from proteins destined to the basolateral surface (in blue). Concomitantly, sphingolipids and phosphatidylcholine become segregated. This model proposes that the two events are coupled. Glycosphingolipids (in light red) cluster together in a process facilitated by cholesterol molecules (in green) intercalating between the hydrocarbon chains of the sphingolipid molecules. These sphingolipid–cholesterol clusters behave like rafts (the red molecules) in the fluid bilayer. The apical proteins have specific affinity for these lipids and are incorporated in these rafts. The basolateral proteins instead are segregated in the regions of the fluid bilayer rich in phosphatidylcholine (in blue).

(Yoshimori *et al.*, 1996). The difference between the plasma membranes of epithelial cells and fibroblasts is that in the former cells the sphingolipid–cholesterol rafts are preferentially segregated to apical domain, whereas in the latter cells the rafts float about in a continuous fluid bilayer.

Evidence indicates that there are two trafficking circuits connecting the Golgi complex with the plasma membrane — one employing the SNARE/Rab mechanism and another using sphingolipid–cholesterol rafting. This raises new possibilities for subcompartmentalization of protein action in the plasma membrane endosome and the Golgi complex. Previously, the lipid bilayer was considered to be a uniform solvent for membrane proteins. The sphingolipid–cholesterol rafts concept imposes organization into the two-dimensional liquid. Proteins could preferentially associate with the rafts or be excluded into the phosphatidylcholine-dominated fluid regions of the bilayer. Not only membrane trafficking but also processes such as cell surface signaling through hormones and growth factors have been demonstrated to make use of these lipid microdomains for increasing specificity and efficiency. If cholesterol is removed artificially from the membrane, the sphingolipid rafts with their associated proteins disintegrate and cannot perform their functions. The raft-stabilizing function of cholesterol is probably its most important cellular function.

Evidence also indicates that disorders leading to deranged cholesterol metabolism and atherosclerosis as well

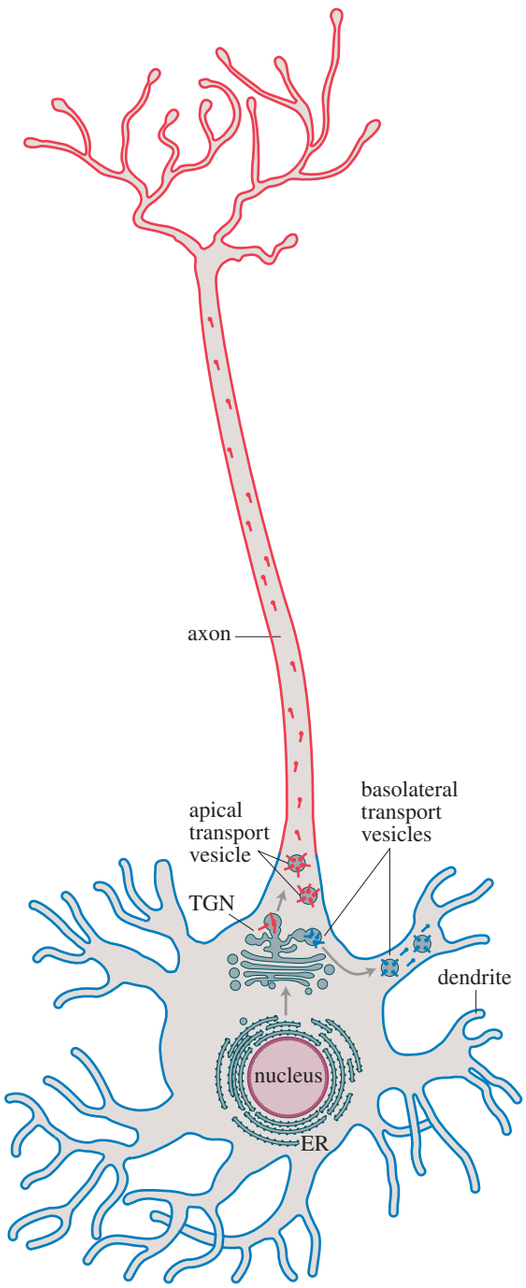

FIGURE 12 The biosynthetic pathway in a neuron. Like epithelial cells, neurons have two domains, the dendrites and the axon — structures which are specialized to ensure the transmission of information from one cell to another. The contact site between the axon of one neuron and the dendrite of another neuron is called the synapse. This specific function requires the delivery of distinct sets of proteins to the axon (in red) and to the dendrites (in blue). The segregation of the trafficking routes in the biosynthetic pathway occurs in the TGN.

as to brain dysfunction seen in Alzheimer's disease and in prion-related diseases (scrapies) involve the functioning of sphingolipid–cholesterol rafts. The rafts ensure fidelity and proper processing of key molecules involved in regulation of processes in the brain that are defective in these disease states. It is hoped that further research will provide new insight into effective remedies for these devastating diseases.

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