

Biological Membranes Architecture and Function

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1. Introduction

1.1. Why are biomembranes a playground for physicists?

Life in all its diversity became possible after nature had found the trick with the membrane. It enabled the separation of living entities from the lifeless and hostile environment under preservation of selective material exchange between the two worlds. It led to the evolution of cells the function of which depends on the well controlled interplay and material exchange between compartments performing different functions. Simultaneously, the biomembranes developed into sites of essential biochemical functions, such as protein biosynthesis or oxidative phosphorylation. The reduction to two dimensions increased the efficiency drastically and opened the possibility for irreversible charge separation (as in the charge transfer chains of mitochondria or photosynthetic membranes) and transient storage of energy in the form of electrochemical potential gradients.

Biomembranes fascinate physicists for several reasons:

- (i) They are examples of two-dimensional colloidal systems exhibiting various novel physical properties (e.g., non-classical elastic properties) which are simultaneously essential for their biological function.
- (ii) Their composition involves about a hundred components and thus poses a real challenge for the development of new concepts of the physical basis of self-organization of multi-component systems.
- (iii) Despite their complexity they allow us to explore the interplay between biochemical modulations of the physical properties of biomaterials and the control of biological functions (e.g., in the course of signal transduction processes).
- (iv) By reconstitution of model membranes from a few lipids and membrane proteins, specific membrane function can be studied on a molecular level.
- (v) Studies of biomembranes yield direct insight into the possible role of universal physical properties for the behaviour and function of biological materials (such as scaling laws or logarithmic laws typical for two-dimensional systems).

For the above reasons, artificial and biological membranes have become a basic topic within the new field of complex fluids.

There is a second motivating aspect. It is hoped that we learn to exploit the tricks of nature for biotechnical applications. Examples are the use of vesicles for drug delivery systems or the combination of membranes with electronic or optoelectronic devices in order to build biosensors.

In the present introductory section the basic principles of the molecular design of biomembranes and some of their fundamental functions are introduced. It is also an attempt to point out that universal physical properties can play a role in biological functions. Examples are the entropy driven repulsion forces and their role

for bioadhesion or the role of membrane bending energy for the stabilization of cell shapes or for shape transitions.

This chapter is considered as an introduction in the field and for that reason only a few references are given for further reading where further references can be found.

1.2. Biomembranes enable the modular design of cells

Cells exhibit a modular design. They are made up of compartments which are specialized for one or several well defined functions. The most important functional compartments of eucaryotic cells are exhibited in fig. 1.

Clearly, the modular design was an essential evolutionary step in order to create some order within the cell and to facilitate the control of such a complex machine. Some important consequences and advantages of this design principle are:

- The intracellular space is divided into two sub-spaces: the lumina of the various organelles and the cytosol.
- An enormous number of different classes of molecules are distributed among these different subspaces, thus reducing the number of directly interacting molecular species.
- It allows the formation of a remarkable gradient of composition between the nucleus and the plasma membrane which is essential for the directed flow of newly synthesized material from the endoplasmatic reticulum to the plasma membrane or the extracellular space as well as for the trafficking of nutrition molecules in the opposite direction.
- Different ionic compositions or pH's can be established in the lumina of the various organelles which is essential (i) for the establishment of electro-chemical gradients across the membranes, (ii) the control of the activity of specialized proteins (such as the digestive enzymes of lysosomes) and (iii) the accumulation of specific proteins within the various subspaces.

2. Molecular architecture of biomembranes

2.1. Cell plasma membrane as three layered composite system with associated macromolecular network

As indicated by the schematic view of fig. 2, the plasma membrane of animal cells is in general a three layered compound system [1–3]. Its center is formed by the liquid crystalline lipid/protein layer. The outside is covered by the glycocalix: a macromolecular film formed by the oligosaccharides of the glycolipid head groups and the branched polypeptide/oligosaccharide head groups of the glycoproteins. As shown in fig. 3, the glycocalix may protrude several tens of nm into the extracellular space. At the intracellular side, the bilayer couples to the membrane associated cytoskeleton: a quasi-twodimensional macromolecular network. A model of its structure is shown in fig. 6. In most cells the inner leaflet of the membrane is coupled to the three dimensional macromolecular network of actin filaments. This may be mediated by the membrane associated cytoskeleton or by other coupling proteins such as talin [18]. For a more detailed discussion of the cytoskeleton-membrane

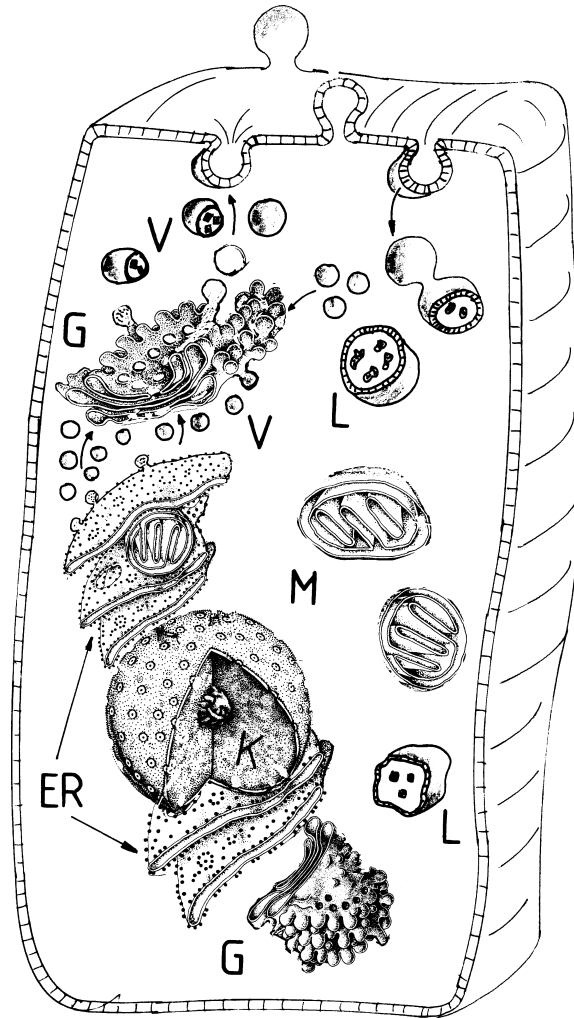


Fig. 1. Modular design of the cell. Schematic view of eucaryotic cell composed of modules with well defined functions. These include: The nucleus, N, (the site of information storage); the endoplasmic reticulum, ER, with associated ribosomes (the location of protein and lipid synthesis); the Golgi apparatus, G, (serving the modification and sorting of newly synthesized proteins and lipids and their directed distribution to other compartments or membranes); the mitochondria, M, (organelles where ATP is produced); the lysosomes, L, (specialized for intracellular digestion). V denotes a whole palette of vesicles (e.g., endosomes) which are required for the molecular transport within the cell and between the cell and its environment. The plasma membrane (PM) forms a selective filter and regulates communication between cells. The intracellular compartments are embedded in the cytoskeleton: a soft network of protein filaments (not shown). The cytoskeleton helps to establish some order within the cytoplasm and, together with the plasma membrane, determines the mechanical stability of the cell. The membranes create three subspaces: the lumina inside of the compartments, the cytosol which is the zone between the compartments, and the extracellular space.

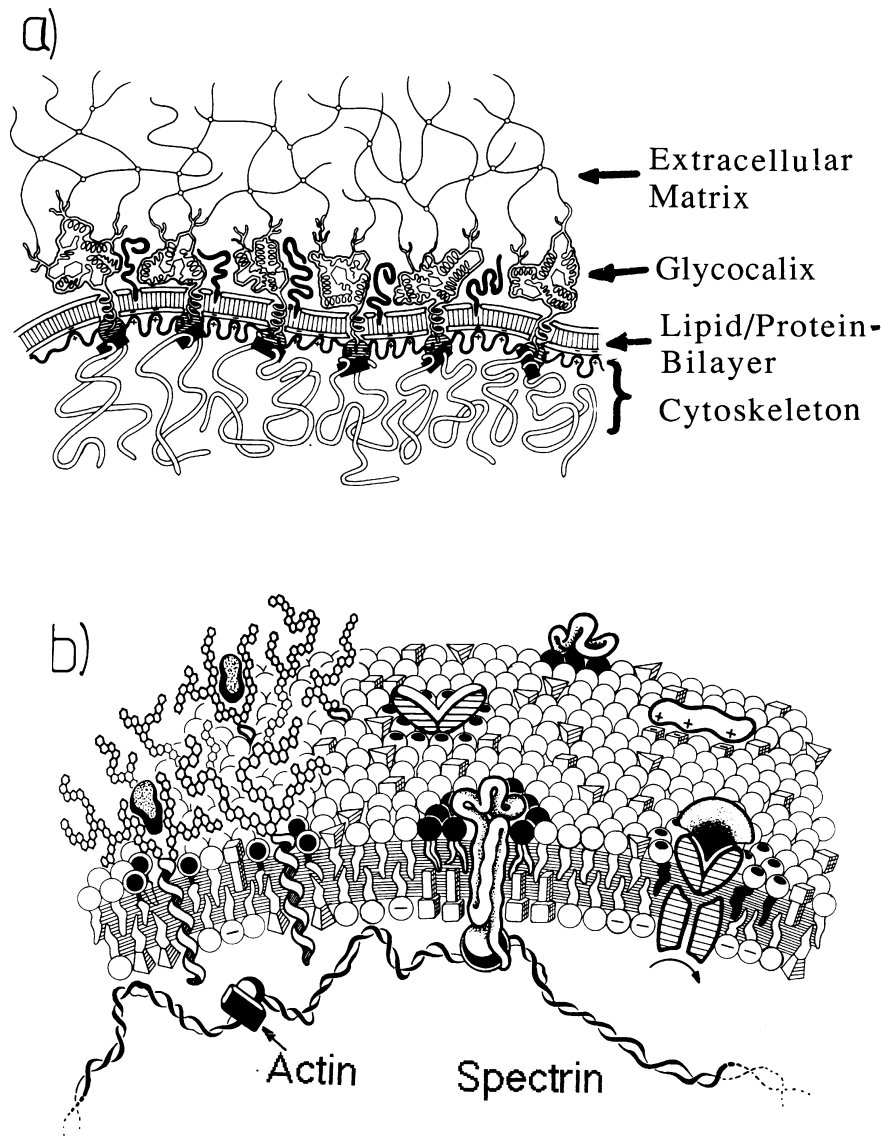


Fig. 2. Coarse and fine structure of plasma membranes: a stratified composite material. a) Schematic coarse grained structure of cell plasma membrane and associated networks. The caricature shows the three layered composite build-up from the lipid/protein bilayer forming the center, the glycocalix facing the extracellular space and the bilayer-coupled cytoskeleton facing the cytosol. The latter may again closely couple to the three-dimensional actin network and the glycocalix may attach to the extracellular matrix (e.g., a collagen IV network). Note that the lipid/protein bilayer and the glycocalix are enlarged in thickness by a factor of about 300 compared to the macromolecular networks. b) Simplified high resolution cartoon of the plasma membrane of an erythrocyte with associated spectrin-actin network. Note the asymmetric distribution of the lipids between two monolayers and that the proteins are surrounded by clouds of specific lipid. For details of the fine structure of cytoskeleton see fig. 6.

Fig. 3. Transmission electron micrograph of the plasma membrane of lymphocytes. The roughly 50 nm thick glycocalix is visualized by staining with ruthium red. (Source: R.D.Dyson, Cell Biology, Allyn and Bacon, 1978.)

coupling see the article by Janmey. In cells of the body tissue the glycocalix couples to the macromolecular network of the extracellular matrix.

The plasma membrane plays many roles:

- The bilayer together with the glycocalix forms a selective filter which controls the transfer of ions, molecules, molecular aggregates and even large particles (such as viruses, bacteria or other cells) between the extracellular space and the cytosol.
- The bilayer is a multifunctional system which can simultaneously be the site for energy producing processes (such as glycolysis) and for the hormone signal transduction and amplification.
- The glycocalix acts as receptor for extracellular signals and mediates the communication between the cell interior and its environment.
- Another function of the glycocalix is to form a connecting link to the extracellular matrices, such as the connective tissue of epithel cell layers of animal cells or the cellulose fibers of cell walls of plant cells. In the former case the coupling is mediated again by receptors, e.g., for collagens or fibronectin.
- The lipid protein bilayer together with the cytoskeleton is responsible for the unique combination of flexibility and mechanical stability of cells.

2.2. Erythrocyte birth and membrane structure

The most thoroughly studied prototype of a compound membrane is the humane erythrocyte plasma membrane [4–9]. It exhibits only a membrane associated cytoskeleton. Since the cell is not nucleated and thus cannot manipulate the membrane composition by genetic expression it is an ideal model to study the fundamental physical properties of stratified composite membranes. Our present view of the

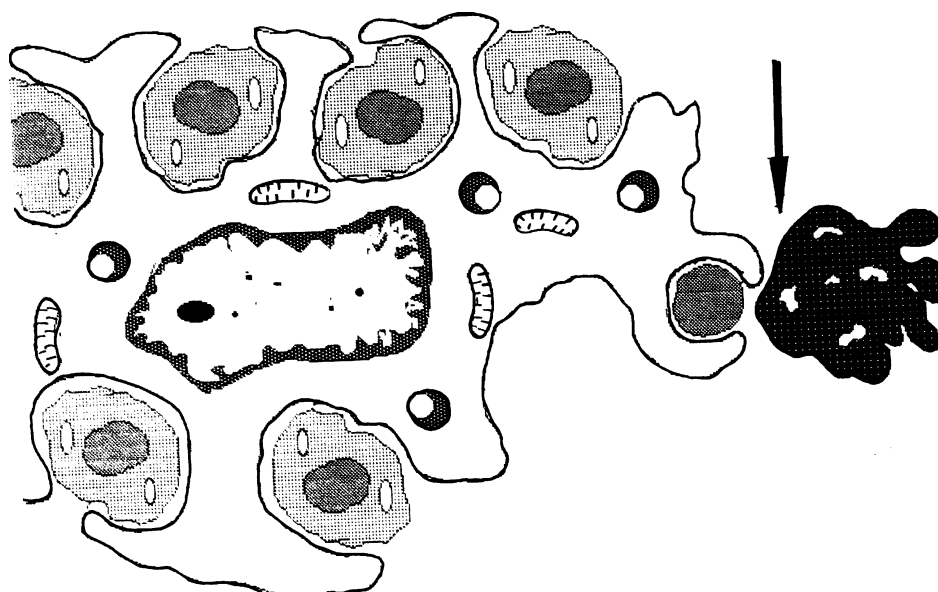


Fig. 4. Formation of erythroblast (red blood cell with nucleus) from megacyte and simultaneous expulsion of the nucleus (cf. arrow) which is taken up by the mother cell (cf. fig. 22 below).

membrane structure is summarized in figs 4 to 7 (cf. references [1], chapter 13, [3], chapter 6).

Birth and maturing. Erythrocytes are formed in the bone marrow by division of giant mother cells (called megacytes or erythrocyte colony forming units) a process triggered by the hormone erythropoetin (compare fig. 4 and reference [3], chapter 16). In the process of detachment, the nascent cell, called erythroblast, loses the nucleus together with part of its plasma membrane by budding (see fig. 24a). The bulk of the cell contains most of the elements of the cytoskeleton but also some other intracellular organelles (e.g., mitochondria and m-RNS for hemoglobin synthesis). This so called reticulocyte undergoes a maturing process during which the mitochondria and the other organelles are degraded and secreted (cf. fig. 24b) while the cytoskeleton assembles beneath the plasma membrane [5]. The initially crumpled cell assumes its beautiful discocyte shape. The matured erythrocyte retains, however, the enzymes required for ATP-production via glycolysis which is the major energy production pathway and the removal of oxidation products such as peroxidases. Most remarkable is the high hemoglobin content of 0.4 mg/ml (corresponding to a concentration of 5×10^{-3} M). Important data concerning the lifetime, production rate and performance of the erythrocytes are summarized in fig. 5.

The lipid/protein bilayer. The bilayer is a two-dimensional smectic A multicomponent alloy shown in fig. 2b. Besides the ion pumps, the major integral membrane proteins are band III, acting simultaneously as anion exchange system and anchoring

Life data of human erythrocytes

Production rate	$2.4 \cdot 10^6$ per sec
Daily loss	$2.1 \cdot 10^{11}$ cells
Total number of RBC's	$2.5 \cdot 10^{13}$ cells
Distance travelled during 120 day life-time	400km
Cell weight	$3 \cdot 10^{-11}$ g
Cell surface	$140\mu\text{m}^2$
Cell volume	$110\mu\text{m}^3$

Fig. 5. Some life data of erythrocytes. After Bessis, *Living Blood Cells and Their Ultrastructure*, Springer-Verlag, 1973; P.G. Fricke, *Schweiz. Med. Wochenschr.* **91**, 1245 (1961).

site for the cytoskeleton, and three classes of glycophorins (A, B and C) which carry about 80% of the oligosaccharides of the red blood cell. Their exact biological role is still unknown. Together with the glycolipids they could be involved in immunoreactions and form a protective layer preventing the direct access of molecules to the lipid bilayer. Their high content of sialic acid residues (about 36 per molecule) are responsible for the high negative surface charge of the erythrocytes which could play a role in the control of the adhesion of the cells to the inner surfaces of the body tissue. The membrane also contains, however, adrenergic hormone receptors and adenylatecyclase, the role of which is still unclear.

The spectrin/actin network (cytoskeleton, [7]). An electron micrograph of the cytoskeleton is presented in fig. 6a [8, 9]. Figures 6b and 6c show a schematic view of the two dimensional organization of the cytoskeleton and its coupling to the membrane. As suggested by the electron microscopic observations [8, 9] the network exhibits an astonishingly well defined hexagonal symmetry (cf. fig. 6a). The network exhibits a remarkable degree of bond orientational order of the triangular lattice. However, the electron micrographs reveal also a considerable number of pentagons and heptagons. These can be considered as -60° and $+60^\circ$ disclinations of the triangular lattice (cf. chapter 5). In addition one observes a substantial number of free filaments as indicated in the schematic view. The network is coupled to the lipid-protein bilayer by coupling proteins as indicated in fig. 6c.

Let us now consider the molecular details of the cytoskeleton. The major constituents of the cytoskeleton are spectrin, actin, ankyrin (also called band 2.1), band 4.1 and tropomyosin. As shown in fig. 7 spectrin is a remarkably flexible protein filament of 100 nm total length. In aqueous solution it forms an elongated random coil of about 35 nm length. It is composed of two chains: an α -chain comprising 2100 amino acids (260000 Dalton) and a β -chain of 1950 residues (250000 Dalton). As predicted by probabilistic considerations (e.g., Chou-Fasman rule) both chains exhibit the beaded-chain structure shown in fig. 7 [6]. The repeating domains (or beads) are expected to be composed of three parallel helices. They are highly

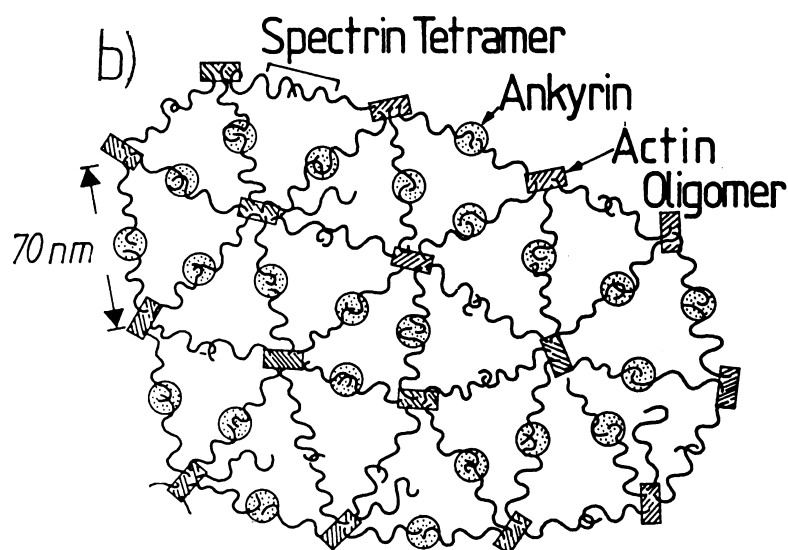


Fig. 6. Structure and membrane coupling of the spectrin/actin network. a) Electron micrograph of spectrin/actin network of human erythrocytes spread on carbon film (?). The lipid/protein bilayer was removed by Triton and the area is extended by about 30% with respect to the natural membrane. Note that some residual ankyrin (thick arrow) and band 4.1 (thin arrow) is still bound. (Source: S.C. Lui et al., *J. Cell Biol.* **104**, 527–535 (1987).) b) Lateral organization of the spectrin/actin network of erythrocytes as suggested by electron micrographs of the cytoskeleton separated from the lipid/protein bilayer by Triton treatment. The spectrin tetramers (head-to-head associated dimers) form the sides and the actin oligomers the corners of a triangular network with a remarkable bond orientational order. Note that the triangular network exhibits some $+60^\circ$ and -60° disclination, reminiscent of a hexatic network and an unknown number of dangling bonds.

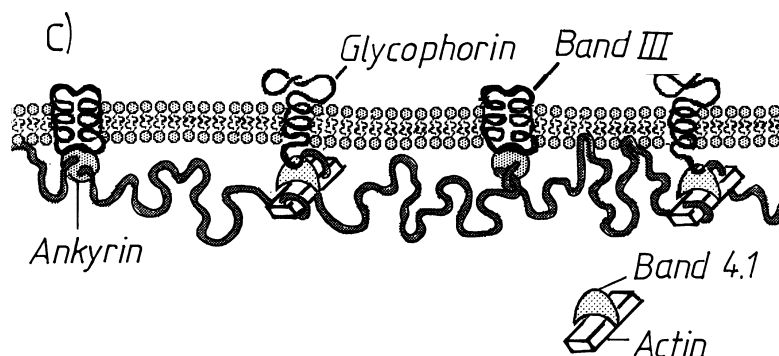


Fig. 6 (continued). c) Coupling of spectrin-actin network to lipid/protein bilayer. Well established is the coupling of the spectrin tetramers (near the bivalent junction) to band III via ankyrin and of the multivalent actin junction via band 4.1 to glycophorin. A further possibility suggested by model membrane studies is in the electrostatic binding of spectrin to acidic lipids (PS and PI) associated with a partial penetration of flexible side chains into the bilayer.

charged, containing typically 22 acidic and 24 basic groups, and are interconnected by flexible links comprising some 15 to 20 amino acids.

The spectrin dimer is a polar molecule since the two chains associate in a parallel way. One end of the dimer has a strong tendency to associate with a second spectrin heterodimer in a head-to-head fashion so that a symmetric $(\alpha\beta)_2$ -tetramer forms. This bivalent cross-link also exhibits a binding site for ankyrin. The other end exhibits binding sites for actin and band 4.1 molecules.

The F-actin oligomers consist of about 13 actin monomers and are about 35 nm long. It is believed that this length is stabilized by association with tropomyosin. This is also a two stranded filament (each chain of molecular weight 35 kD) of 35 nm length and the number of species per cell agrees well with that of actin oligomers, see table 1.

Table 1

Relative number of essential elements of membrane associated cytoskeleton of erythrocytes. For a given area A of the cell membrane a triangular lattice composed of $n_t = 4A\sqrt{3}\langle L^2 \rangle$ triangles, $n_b = 3/2n_t$ bonds and $n_v = n_t/2$ vestiges can be formed where $\langle L \rangle$ is the average end-to-end length of the spectrin tetramer. For the average membrane area of $\langle A \rangle = 140 \mu\text{m}^2$ the above number of spectrins yields a lattice constant of $\langle L \rangle = 72 \text{ nm}$. A freeze fracture EM suggests a value $\langle L \rangle = 75 \text{ nm}$ (data from ref. [17]).

Protein	Band III	Glycophorin	Spectrin Tetramers	Actin Oligomers	Tropo-myosin	Ankyrin	Band 4.1
Number of Copies	2.5×10^5	A 2×10^5 B 7×10^4 C 4×10^4	1×10^5	3-4 $\times 10^4$	4×10^4	1×10^5	2×10^5
Phosphorylation sites	—	—	5	—	8	—	—

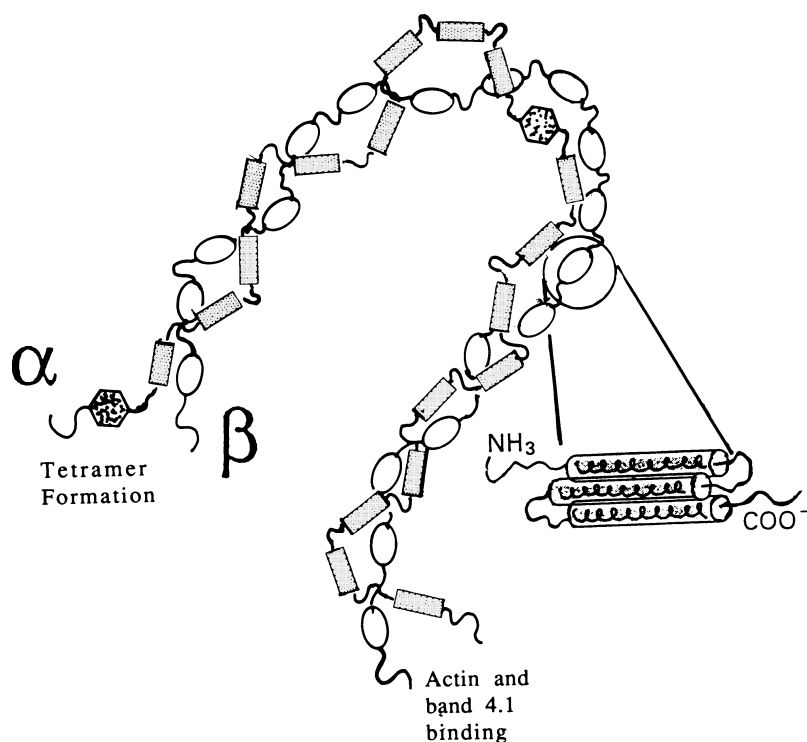


Fig. 7. Molecular model of spectrin dimer composed of two polypeptide chains, α and β , which are at least partially twisted around each other. Both the α and β chains are built-up of repeating domains of 106 amino acids which most likely form a triple stranded arrangement of α -helices (cf. inset) and are interconnected by flexible segments of variable length, typically consisting of 17 amino acids. The α -chain is composed of 20 and the β -chain of 18 domains. Note the different structure of the 9th and 20th domain of the α -chain (indicated by hexagons). (After D.W. Speicher, *J. Cell Biochem.* **30**, 245–256 (1986).)

Ankyrin, one of the cytoskeleton-bilayer coupling proteins, is a 200 kD protein consisting of two domains. One can bind specifically to a domain of the β -chain of spectrin which is located near the spectrin-spectrin association site. The other can bind to the cytoplasmic domain of band III.

Band 4.1 (a 82 kD protein) exhibits one binding site for actin and one for the cytoplasmic domain of glycophorin A [7]; but it can also bind to band III. For that reason, it is considered as the second membrane anchoring protein of the cytoskeleton.

The number of spectrin tetramers (1×10^5) and actin oligomers ($3-4 \times 10^4$) found per cell is just sufficient to form a triangular network of about $\langle L \rangle = 70$ nm bond length in a cell exhibiting a surface area of $140 \mu\text{m}^2$ (cf. table 3 and Zilker et al. [10]). The total number of band 4.1 molecules per cell is about $\sim 2 \times 10^5$ and coincides with that of the spectrin tetramers corresponding to a 5–6 fold excess with respect to actin oligomers. In fact one major role of band 4.1 is to facilitate (together with adducin) the spectrin-actin association, see fig. 6.

Concerning the coupling of the network to the lipid/protein bilayer, it is well established that it is mediated (1) by ankyrin which couples the bivalent junction to band III proteins and (2) by band 4.1 which may attach the multivalent cross-links to the carboxyl-terminal of glycophorin C. The number of ankyrin molecules is about 10^5 and agrees indeed with the number of spectrin tetramers. The attachment of the cytoskeleton to the bilayer is further mediated by band 4.1 which is supposed to couple the multivalent cross-link preferentially to the glycophorin A. However, coupling to band III is possible in the absence of glycophorin A which may occur as a result of hereditary diseases.

For the small deformations associated with the membrane flickering the spectrin/actin network exhibits a much higher degree of softness than expected from its mesh size and the elastic modulus of spectrin [10]. It appears that a considerable fraction of the ATP-consumption of red blood cells is required to maintain this softness. Spectrin and the major regulating coupling proteins (ankyrin, band 4.1, tropomyosin) exhibit one or several sites for phosphorylation. Following Bennet [7], phosphorylation reduces the binding (1) of band 4.1 to spectrin by a factor of five and (2) of ankyrin to both band 3 and spectrin by a factor of five. In contrast, phosphorylation of spectrin has only a slight effect on the spectrin-spectrin and the spectrin-actin binding. This suggests that the coupling of the networks to the lipid/protein bilayer is modulated by phosphorylation/dephosphorylation reactions while the lateral association is only slightly affected.

There is growing evidence that other cells possess similar membrane-coupled cytoskeleta. An established case is the thrombocyte which contains a high content of a 200 nm long spectrin-like filament called fodrin. Other likely examples are nerve cells where spectrin-like molecules are abundant. It is thus well possible that two dimensional networks similar to that of erythrocytes mediate the coupling of the 3D-actin network of the cytosol to the membrane.

3. Molecular composition of the lipid/protein bilayer

3.1. Universalities and peculiarities of lipid composition

Fortunately, only a few classes out of the enormous variety of possible lipids are used by nature to build up animal cell membranes (see fig. 8). These may be further divided into a subgroup playing a predominantly structural role and another subgroup with mainly a functional role.

To the first subgroup belong (1) cholesterol, (2) the four major classes of phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomyelin (SPHM) (cf. list of abbreviations) and (3) cerebroside which are analogues of SPHM with the phosphocholine group replaced by non-charged sugars such as galactose or glucose. A special type of phospholipid which is only present in mitochondria is the four-chain lipid cardiolipin. This lipid is formed by fusion of the two phosphatidic acid molecules and exhibits four negative charges at neutral pH.

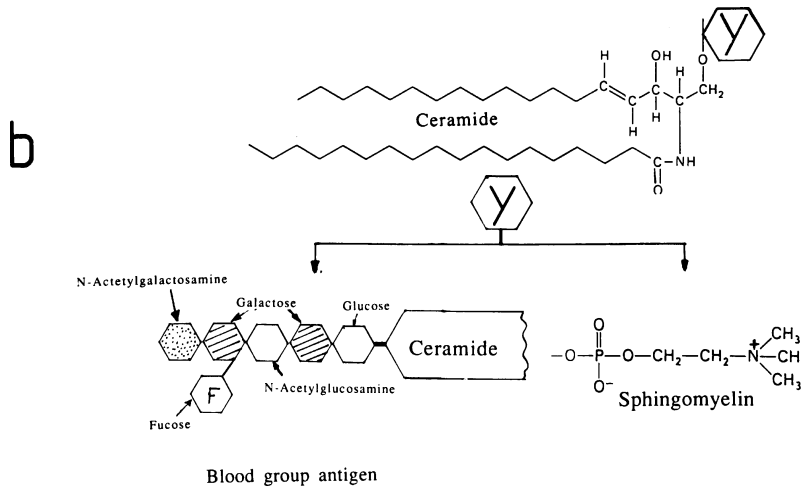
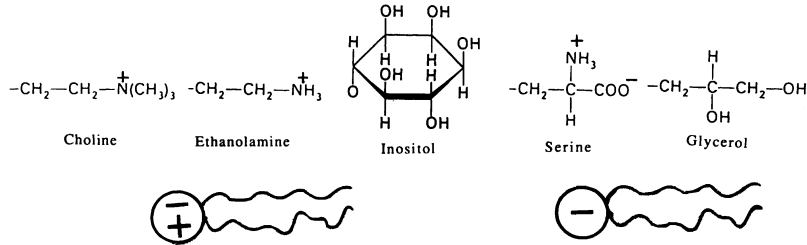
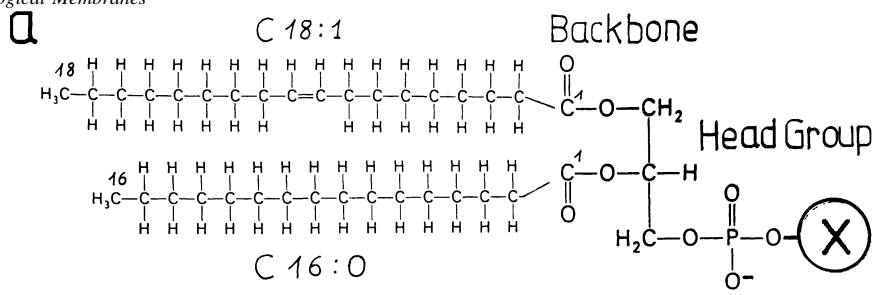


Fig. 8. Major classes of membrane lipids of animal cells. The major classes of two-chain lipids are distinguished by the structure (i) of the fatty acid chains (number of C-atoms and double bonds), (ii) the semipolar backbone and (iii) the head group. The phospholipids (a) exhibit a glycerol-backbone while that of sphingomyelins and glycolipids (cerebrosides, gangliosides) is a so-called sphingosine (b). The four major classes of phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) differ only in the hydrophilic group esterified to the phosphate group. The sphingomyelin (SPHM) exhibits a phosphocholine head group (as PC), the cerebrosides a single sugar residue (glucose, galactose) and the gangliosides a complex (branched or linear) oligosaccharide. The ganglioside shown in (b) represents the antigen specifying blood group A.

Note: Each two-chain lipid may be transformed into a one chain lipid (lyso lipid) and fatty acid.

To the lipids with a functional role belong (1) phosphatidylinositol as precursor of the 2nd messengers based on phosphoinositols, (2) phosphatidic acid and phosphatidylglycerol (e.g., as intermediates of lipid synthesis) and many types of gangliosides (a member of the cerebrosides) functioning as carriers of blood group antigens. In this context one should also mention another type of functional amphiphile: the dolicholphosphate [3] which acts as carrier of oligosaccharides during their attachment to gangliosides or glycoproteins.

The membranes of animal cells also contain a substantial amount (several mole%) of neutral lipids such as diacylglycerols (glycerol with two attached fatty acid chains) and triacylglycerols (glycerol with three fatty acid chains) in addition to fatty acids and lyso-phospholipids. These types of amphiphiles are most probably mainly metabolic intermediates or are transiently formed during metabolic processes where lipids play an essential role. Examples are (1) the synthesis of prostaglandins: hormones exhibiting many regulatory functions for which arachidonic acid is a precursor or (2) the (hormone) signal transduction processes involving poly-phosphoinositols as second messenger (cf. chapter 17).

The membranes of plant cells (in particular seeds) contain the same types of phospholipids as animal cells. The most abundant phospholipid constituent is, however, phosphatidylglycerol in leaves. Other important constituents are the neutral digalactosides and the negatively charged sulfolipids. In thylakoid membranes these comprise 40% and 4%, respectively.

A completely different class of amphiphiles used by nature for membrane design are the bipolar lipids (also called bola lipids) of archaebacteria.

A good overview of the different classes of lipid is given in reference [2].

3.2. *Membranes of cellular organelles exhibit characteristic distributions (patterns) of lipids*

From the physical point of view lipids are characterized by three structural features (cf. fig. 8):

- the size and electrical property of the head groups which may be charged (as PI, PS, sulfolipids and some gangliosides), zwitterionic (as PC, PE, SPHM) or neutral (as cerebrosides or galactosides)
- the number of carbon atoms (varying between 16 and 24 for the most abundant fatty acids) and the number of double bonds (ranging from 1 to 6)
- the structural difference between the two alacyl chains of each lipid

Considering both the head group and the chain structure, biomembranes are composed of an astonishingly large number of different types of lipids. For erythrocytes this number is about 100. Therefore, one of the major questions of membrane research is: why does nature use so many types of lipids? Is it a left-over of evolution or a structural and functional necessity?

One primary aim of the physics of membranes must be to find answers to these questions. This requires, however, a better understanding of the basic physical properties of multi-component systems as well as of the mechanisms of specific lipid-protein interactions. These topics will be treated in chapters 5 and 7.

Table 2
Summary of lipid composition of various cellular organelles of mammalian liver cells and erythrocyte plasma membrane. Values are given in percentages by mass of total lipids. (Source: Jamieson and Robinson, [4].)

Compartment Lipid	Plasmamembrane		Endoplasmatic Reticulum	Golgi	Lysosome	Nuclear Membrane	Mito- chondria	Nerve Cells	
	human erythrocyte	rat liver						Neurons ⁽⁵⁾	Myelin ⁽⁶⁾
phosphatidyl- cholin	20	18	48	25	23	44	38	48	11
phosphatidy- ethanolamine	18	12	19	9	13	17	29	21	17
phosphatidyl- serine	7	7	4	3		4	0	5	9
phosphatidyl- inositol	3	3	8	5	6	6	3	7	1
sphingomyelin	18	12	5	7	23	3	0	4	8
cardiolipin	—	—	—	—	≈ 5	1	14	—	—
glycolipid	3	8	traces	0		traces	traces	3	20 ⁽⁷⁾
others	11	21 ⁽¹⁾	10 ⁽²⁾	43 ⁽³⁾	16 ⁽⁴⁾	15	13	1	6
cholesterol	20	19	6	8	14	10	3	11	28

Footnotes: ¹⁾These include 6% free fatty acid, 2.5% lyso PC, 2.5% cholesterol esters, 7% triglycerides.

²⁾These include 5% triglycerides.

³⁾These include 10% triglycerides, 18% free fatty acid and 5% cholesterol esters.

⁴⁾These include 3% triglycerides and 8% cholesterol esters.

⁵⁾Average lipid composition of rat brain neurons.

⁶⁾Bovin brain myelin.

⁷⁾Essentially ceramides.

In table 2 the lipid compositions of plasma membranes and some intracellular organelles are summarized. These exhibit some remarkable universal features but also characteristic differences between the various organelles.

- The lipid composition of plasma membranes of mammalian cells is remarkably similar (column 1 and 2 of table 2). This holds in particular for the high content of cholesterol which amounts roughly to 20 weight % (or 50 mole %) of the total lipid. A similarly high cholesterol content is also characteristic for myelin membranes (about 25 weight %). The cholesterol content of the inner compartments is considerably lower, with the exception of lysosomes.
- The differences in lipid composition of plasma membranes and the inner organelles is small but quite remarkable and characteristic. Most remarkable are: (1) the astonishingly low cholesterol content in the endoplasmatic reticulum (10%) and Golgi membranes (8%) but also in the inner and the outer membrane of mitochondria ($\leq 5\%$); (2) the similarity in cholesterol content of lysosomes and the plasma membrane; (3) the low content of sphingomyelin in the nuclear membrane (3%) and the ER (ca. 5%); (4) the high SPHM-content of the plasma membrane (ca. 24% in humane erythrocytes) and in the lysosomes (23%); and (5) the high PC content of the nuclear membrane.
- The content of charged lipids is roughly 10 mole % in all membranes and is highest in the plasma membranes (11–13%). There are, however, remarkable differences in the PS:PI ratio. Thus PS is reported to be very low in the lysosomal and the smooth ER-membrane [4] where it is replaced by PI.
- The lipid composition of both mitochondrial membranes is remarkably different from that of the other organelles, a consequence of the partial genetic (and biosynthetic) independence of this organelle. Thus mitochondria contain 25 weight % of charged lipid in the inner and 19 weight % in the outer membrane. The major charged component in the former is cardiolipin and in the latter PI.
- The glycolipids (neutral glycolipids with up to 15 neutral sugars and the charged gangliosides; cf. fig. 8) reside almost exclusively in the plasma membrane where they comprise about 3% of the total lipid. The gangliosides contribute essentially to the negative surface charge of cells.

Table 3 presents a summary of the distribution of the most abundant fatty acids among the various phospholipids of humane erythrocytes. Many more data about the fatty acid composition of nerve cells are given in Jamieson and Robinson [12] (volume II).

The chain lengths vary between 18 and 24 C-atoms. The most abundant lengths of the saturated hydrocarbon chains are C 16:0 and C 18:0 (cf. fig. 8 for nomenclature of the chain structure) which comprise about 35% in erythrocytes but some 50% in myelin membranes. The major non-saturated lipids are C 18:1, C 20:4 and C 22:6. The former two species are most abundant in erythrocytes while the nerve membranes of the brain (not myelin) are very rich in the 6-fold nonsaturated lipid.

Most interesting is the different distribution of the fatty acid among the different types of lipids. Thus

Table 3

Distribution of most abundant fatty acids among lipids of humane erythrocyte. The usual nomenclature C (m:n) is used where m is the number of C-atoms and n the number of double bonds. Amounts smaller than 1% are indicated by dashes. Chains with a total relative abundance of < 1 mole% are left out (such as C 20:0 and C 23:0). Therefore the numbers do not add up to 100%. Source: Jamieson and Robinson, [4].

Lipid (mole%)	16:0	18:0	18:1	18:2	20:3	20:4	22:0	22:4	22:5	22:6	24:0	24:1
PC	31	12	19	22	2	7	—	—	2	—	—	—
PE	13	12	18	7	2	24	2	8	4	8	—	—
PS+PI	3	37	8	—	3	24	3	4	3.5	10	—	—
SPHM	24	6	—	3	—	1.4	9.5	—	—	—	23	24
Total	20	17	13	9	1.5	13	2	3	2	4	5	4

- Phosphatidylcholines are mainly composed of short chains (16 to 18 C-atoms) and the main unsaturated components are C 18:1 and C 18:2.
- Sphingomyelin contains, however, an astonishingly high content of long chain lipids with 24 C-atoms with none or only one double bond (50%). This high content of long chains is also found in the sphingomyelin of myelin membranes [12, 16].
- In contrast to PC, phosphatidylethanolamine contains a relatively high content (about 40%) of polyunsaturated chains; in particular C 20:4 (which comprises 24% of all chains).
- The charged lipid components (PS and PI) are also distinguished by a remarkably high content of non-saturated lipids. Thus, they contain a high content of C 20:4 chains (25%).

3.3. The four major subclasses of membrane proteins

From the point of view of lipid/protein interaction the membrane associated proteins may be divided into the following sub-classes (cf. figs. 7, 9 and 10).

A. Proteins interacting predominantly with the hydrophobic core of the lipid bilayer

Presently, the most prominent member of this sub-class is the reaction center of photosynthetic bacteria (since it is still the only protein the structure of which has been determined at Å resolution). Most ion channels as well as ion and molecular pumps belong to this class. As an example we consider the anion exchange protein of red blood cells denoted as band III protein. It is a huge protein of 929 amino acids. Figure 9 shows the distribution of amino acids with hydrophilic and hydrophobic side chains. This so-called hydrophilicity plot shows that the first 420 amino acids counted from the NH₂-terminus penetrate deep into the cytosol, while segments 420 to 900 form the membrane spanning part. The orientation of the final 50 amino acids is still unclear [13].

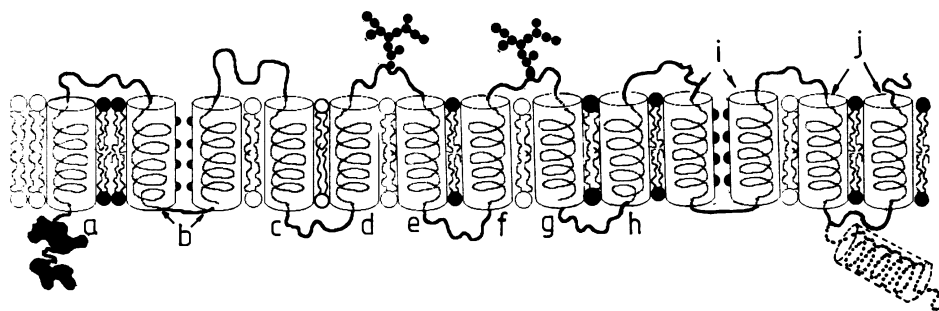
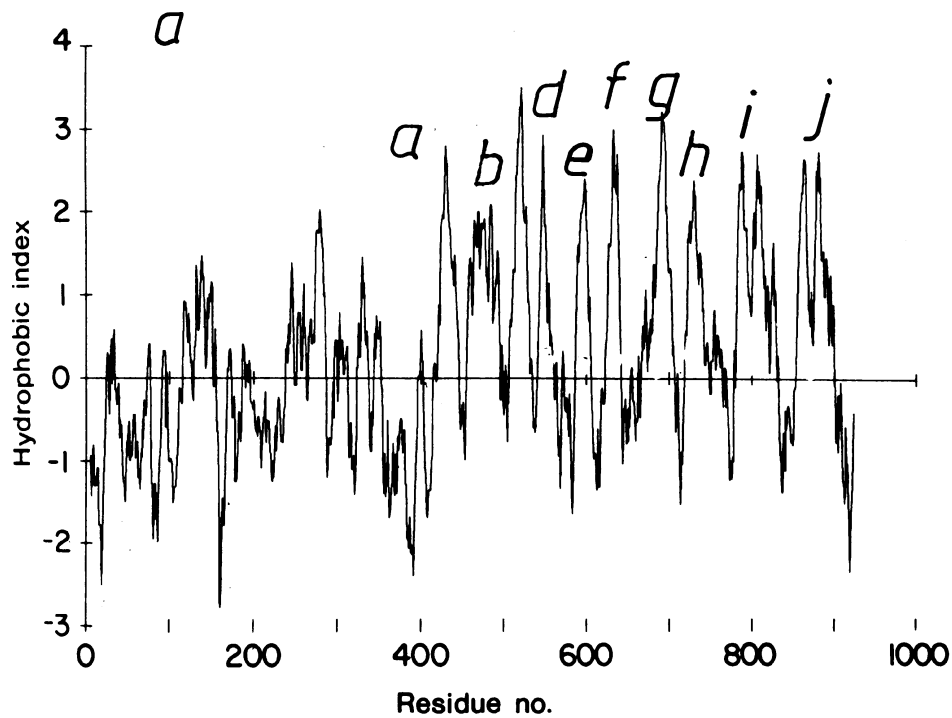


Fig. 9. a) Distribution of amino acids with hydrophobic and hydrophilic side chain along polypeptide chain of anion exchange (band III) protein of erythrocytes (called hydrophobicity plot). The built-up from a predominantly hydrophilic (first 420 amino acids of NH_2 -terminus) to strongly hydrophobic (= membrane spanning) is obvious. The latter has a quasi-periodicity of ten. Source: R.R. Kopito and H.F. Lodish, *Nature* **316**, 234 (1985), [6]. b) Model of molecular architecture of band III protein as suggested by periodicity of hydrophobicity-plot and lengths of the ten hydrophobic stretches of the membrane spanning part. The hydrophobic band *b* comprises 38 amino-acids which can form two antiparallel and amphiphatic helices facing each other with polar surfaces. Also the domain *j* can form an amphiphatic cylinder. The orientation of the last domain (second half of hydrophobic band *j*) is unclear. It could either penetrate the bilayer or extend into the cytosol. Note that one amino-acid contributes 0.15 nm to the length of an α -helix.

The cytosolic domains composed of the first 420 amino acids act as binding sites for the spectrin anchoring protein ankyrin (see fig. 6) but also for hemoglobin and for various enzymes involved in glycolysis.

The hydrophilicity plot exhibits a 10 fold periodicity of the membrane spanning part (band *a* to *j* in fig. 9) suggesting ten membrane spanning domains. However, a closer inspection of the lengths of these domains suggest that the band III molecule penetrates the bilayer with up to 13 helices. Seven of the membrane spanning domains (*a*, *c*, *d*, *e*, *f*, *g*, *h*) comprise 20–24 amino acids which is just sufficient to form α -helices of 35 Å length (note that one amino acid contributes 1.5 Å length). Three of these domains (*b*, *i*, *j*) contain nearly twice as many hydrophobic segments (for instance 38 in the case of band *b*) and thus have to form hairpin-like loops within the bilayer. These could be again α -helices, but four associated loops of β pleated structure would also be consistent with the bilayer thickness. As indicated in fig. 9 the two helices of band *b* could exhibit an apolar and a polar side facing each other with the latter side.

The orientation of the final 50 amino acids (band *j*) is still unclear. They could well extend into the cytoplasmic domain instead of penetrating the membrane as indicated by the dashed helix shown in fig. 9. The loops interconnecting the hydrophobic domains penetrate alternatively into the cytosolic or the extracellular space. Some of the loops carry an oligosaccharide and band III is thus a glycoprotein.

The composition of band III from two huge parts, one of which is situated within the bilayer (integral part) while the other penetrates into the aqueous regions, is typical for multifunctional enzymes. In many cases the extracellular part is non-covalently linked to the integral part. Examples of this type of association are the F_0 -domain of ATP-synthetases (F-type ATPase) or the cytochrome C coupled to the photosynthetic reaction center.

B. Transmembran proteins which are anchored by one hydrophobic stem within the bilayer (cf. fig. 10)

Prototypes of this class are (1) peptide hormone receptors, e.g., for insulin and transferrin, (2) membrane bound antibodies and (3) cell adhesion mediating receptors of the integrin family, e.g., for fibronectin and collagens. Most of the receptors are oligomers of two identical domains such as transferrin and the insulin receptor.

A simple example of this type of integral protein is glycophorin which is exhibited in fig. 10. This glycoprotein recognizes specific plant lectins, such as wheat germ agglutinin, blood group antigens and influenza virus proteins. It is, however, also involved in the coupling of the spectrin/actin network to the bilayer. The membrane spanning part appears to consist of two domains: a very hydrophobic domain (residues 75–95) and a sequence (residues 62–74) which contains two pairs of oppositely charged residues (Glu^- and His^+). The former can just form a bilayer spanning α -helix of 33 Å length. By ion pair formation, the latter could penetrate into the bilayer by forming a hairpin-like loop similar to a β -pleat as indicated in fig. 10. Three other remarkable features are:

- The accumulation of 4 positively charged residues at the segment near the cytoplasmatic monolayer which could strongly couple electrostatically to an acidic phospholipid leading to local lateral segregation of the lipids.

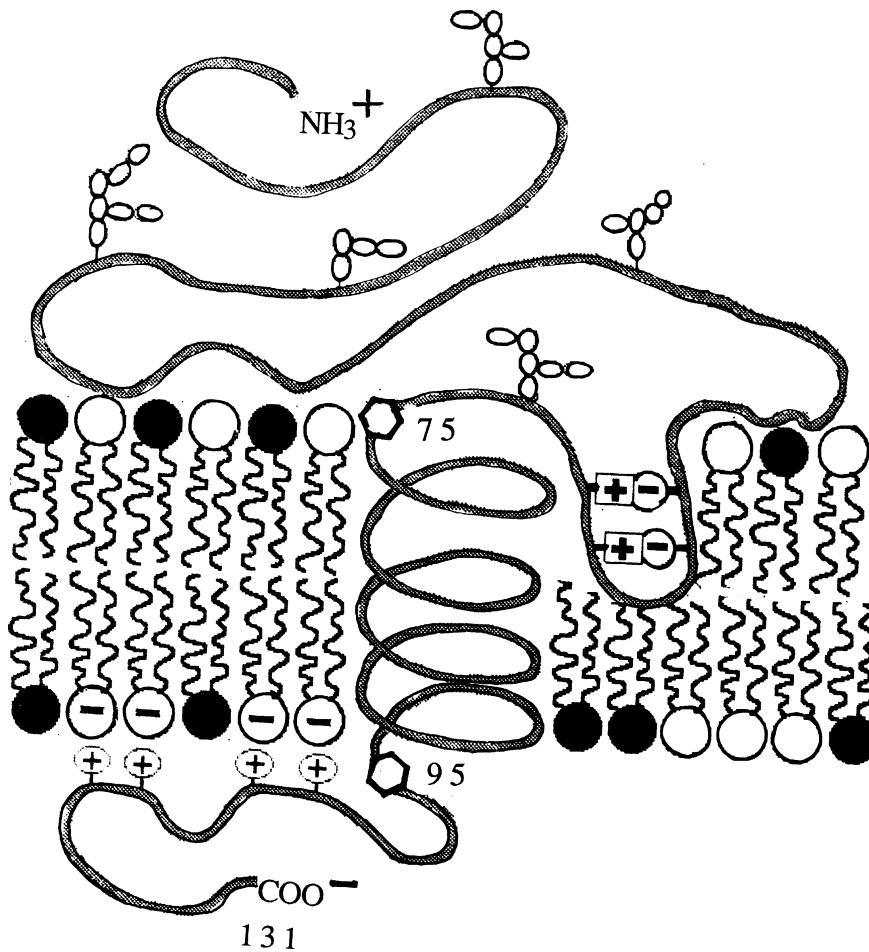


Fig. 10. Schematic structure of glycophorins: an example of a small membrane anchored receptor. The glycoprotein is composed of 131 amino acids with a large head group (61–74 amino acids) pointing into the extracellular space and a smaller (36 amino acids long) –C-terminal extending into the cytosol. Remarkably, the segment formed by amino acids 62 to 74 could form a hairpin-like loop (reminiscent of β -pleated structure) penetrating into the bilayer with the 2 pairs of acidic and basic segments forming ion pairs. The positively charged segments, 96, 97, 100, 101, could link the cytoplasmatic domain to the anionic lipids of the cytoplasmatic monolayer inducing simultaneously local phase segregation (cf. chapter 5).

- The head group carries 16 sugar oligomers containing a high content of negative sialic acid molecules (36 residues) which is expected to lead to a drastic repulsion between the segments resulting in a highly extended conformation.
- Judged from the probabilistic methods such as the Chou–Fasman rule, a large fraction of the extracellular domain (44%) and of the intracellular segment (75%) form random coils and may thus exhibit a rather swollen conformation.

C. Classes of proteins attached to membranes by lipid anchors

One can distinguish three types of anchors.

- One consists of a glycolipid (phosphatidylinositol) to which the protein moiety is coupled via a complex (phosphorylated) oligosaccharide. This type is used to couple enzymes (e.g., phosphatase) to the extracellular sides of membranes. It is, however, most essential for the coupling of cell adhesion proteins to the outer leaflet of plasma membranes. Examples are the adhesion proteins of amoeba and lymphocytes (N-Cam) and mammalian antigens.
- A second group of proteins is anchored by means of fatty acids such as myristic acid (14 carbon atoms). One example is the so called transforming protein, (a viral protein causing cancer); another the α -unit of G-proteins (cf. fig. 26).
- In a third sub-class, the anchor consists of a hydrophobic chain with polyene-like structure (called farnesyl) and the protein moiety is coupled to it by a S–S-bridge between a cystein side group and the $-\text{CH}_2-\text{SH}$ -head group of the hydrophobic anchor. This anchor couples transforming proteins to the extracellular leaflet of plasma membranes. It is also involved in the anchoring of part of of the G-protein (the β/γ -domain) to the monolayers facing the cytosol (cf. fig. 26).

It should also be mentioned that fatty acids (in particular palmitic acid) facilitate the proper coupling of large head groups of membrane receptors to the lipid bilayer. Examples are the transferrin and insulin receptors. In addition, many proteins mediating the coupling of the cell cytoskeleton to membranes carry lipid anchors; although they couple predominantly to the lipid/protein bilayer by non-covalent binding to membrane receptors. One example is talin [18] which together with vinculin mediates the binding of actin filaments to cell adhesion proteins such as integrins.

D. Adsorbed proteins

Model membrane studies suggest that proteins considered as water soluble may strongly interact with lipid bilayers containing charged lipids. Well studied examples are cytochrome C, myelin basic protein and spectrin [14] (cf. also chapter 5). Most remarkable is that these studies reveal a substantial penetration of some flexible loops of the polypeptide into the semipolar surface of the lipid bilayer and that this latter coupling contributes significantly to the protein adsorption. The penetration appears to require, however, a transient local expansion of the bilayer leading to the exposure of its hydrophobic part. Such local expansions could well occur in biological membranes, e.g., during the hormone mediated decomposition of phosphoinositols (cf. chapter 17) leading to the formation of remarkable quantities of diacylglycerol.

3.4. Bilayer asymmetry is an essential feature of the modular design of cells

The creation of three different spaces (the lumina, the cytosol and the extracellular fluid) separated by membranes was an essential evolutionary step towards higher forms of life since it enabled the separation of conflicting biochemical processes

such as biosynthesis and degradation. A prerequisite for this division into functional spaces is the vectorial design of the membranes. Essentially all membrane proteins of a given type are oriented in the same way. Thus, the oligosugar-carrying sides of the glycoproteins appear to extend exclusively into the extracellular or luminal spaces and the glycolipids reside practically all in the lipid monolayer facing these spaces. This vectorial orientation of the integral membrane proteins is paralleled by a remarkable asymmetric distribution of the different classes of lipids. The best studied case is again the erythrocyte plasma membrane [15, 16]. The phosphatidylserines (PS) reside nearly exclusively (that is to 98%) in the inner and sphingomyelins (SPHM) (96%) as well as gangliosides (=glycolipids) (100%) in the outer monolayer. In contrast, the asymmetric distribution of PE and PC is somewhat less exclusive: 75% of PC is in the outer and 80% of PE in the inner monolayer. Cholesterol is expected to be mainly randomly distributed between the two leaflets. The same lipid asymmetry appears to hold indeed for all membranes of the intracellular compartments of nucleated cells, that is PS and PE reside predominantly in the monolayers facing the cytoplasm and PC and SPHM in that pointing towards the extracellular space and the lumen, respectively.

What is the driving force for this lipid asymmetry? Much evidence has been provided that it is powered by specific and possibly non-specific transport proteins (called phospholipid translocases or flippases) although these transporters have not been identified yet. The trans-bilayer dislocation of phospholipids (called lipid flip-flop) in pure lipid bilayer vesicles is extremely slow: that is transfer times between some 12 hours and several days have been reported. In erythrocytes it is much faster: transfer rates are about 4 min for PS 30–40 min for PE and 2–10 hours for PC and SPHM [15]. If integral membrane protein fractions derived from the erythrocyte plasma membranes (or its endoplasmic reticulum) are, however, reconstituted into the lipid vesicles, the lipid translocation occurs with half-times of 15 min to one hour, showing that some of these proteins accelerate the lipid flip-flop.

The phospholipid flippases of the endoplasmic reticulum are required for the transfer of newly synthesized lipids from the cytoplasmic to the luminal side of the bilayer since their biosynthesis is exclusively located in the monolayer facing the cytoplasm. These transporters are not necessarily specific.

The nearly complete PS asymmetry in the plasma membrane of erythrocytes appears to be mediated by a PS-specific translocase which requires ATP for their function. A Mg-dependent ATP-ase, the activity of which is dependent on PS has been identified as a possible candidate [1, 15]. It can be blocked by vanadate.

The real functional role of the lipid asymmetry is not understood yet. The nearly exclusive residence of PS in the cytoplasmic leaflets of all membranes could be important for the coupling of extrinsic cytosolic proteins or of cytoplasmic domains of integral proteins to the inner membrane surface. An example of the latter are glycophorin shown in fig. 10 and transferrin receptors (cf. chapter 5). Many actin binding proteins mediating the coupling of F-actin to the membrane such as hisactophilin or talin [18] carry excess positive charges and interact strongly with PS.

4. Regulation of osmotic equilibrium: an ubiquitous task of membranes

One universal task of membranes is to establish different ionic milieus between the cytoplasm and the extracellular space or the lumina of the cellular organelles, respectively, in order to control the osmotic equilibrium (and thus cell shapes) or to create membrane potentials. For that purpose most membranes contain a whole palette of ion pumps, ion channels and exchange systems for ions and molecules. At first sight, the concerted control of the chemical and electrochemical gradient of the various ions (essentially, H^+ , Na^+ , K^+ , Cl^- , Ca^{++} , Mg^{++}) appears extremely complicated.

- The chemo-osmotic equilibrium of mammalian cells is established by about 20 different types of transporters, not counting the voltage dependent channels for ions such as Na^+ , K^+ .
- The control of such a ‘simple’ system as erythrocytes is governed by more than 20 ion concentrations and ion fluxes.
- The fluxes of the various species are strongly correlated. The effect of a Ca^{++} -influx in erythrocytes can for instance be compensated, at least transiently, by a K^+ -efflux, see section 4.6 below.

4.1. Ion and molecular transport is controlled by only a few classes of pumps

The situation is fortunately simplified by the fact that the variety of electrochemical equilibria is maintained by only a few classes of transporters. These are distinguished by the energy source adopted for driving the ion or molecule translocation against the (electro)chemical potential gradient. Possible energy sources are ATP, electrochemical potentials, and photochemical processes. The most important types of pumps are:

ATP-driven ion pumps

Most pumps involved in ion transport are driven by ATP-hydrolysis. This type is therefore denoted as ATPase. They are again divided into two sub-classes: P-type and V-type pumps. To the P-type class belong:

- The Na^+/K^+ -ATPase shown in fig. 11a. This enzyme establishes the Na^+ - and K^+ -concentration difference between the cytosol of mammalian cells and the extracellular space, and creates the membrane potentials of excitable cells. One particular task is, for instance, to recycle the Na^+ from the tubular fluid in the kidney by pumping the ion from the tubular lumen back to the blood across the epithelial cell layer;
- the Ca^{++} -ATPases which are the primary pumps establishing the low cytosolic Ca^{++} content ($< 10^{-7}$ M) of animal cells and which pump the ions into Ca^{++} -storage vesicles such as sarcoplasmic reticulum vesicles of muscle cells;
- the H^+/K^+ -ATPases which generates the high acidity of the stomach.

The V-type ATPases are essentially proton pumps. They reside in large quantities in lysosomal membranes and provide for the low pH of their lumen by continuously pumping protons from the cytosol into the lumen. They are very large integral

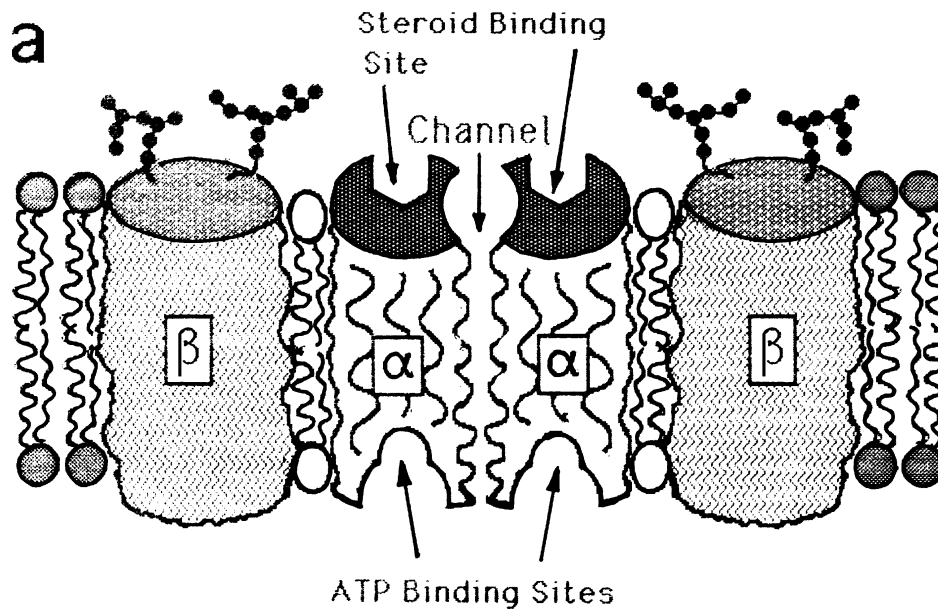


Fig. 11a. Schematic view of quaternary structure of Na^+/K^+ -ATPase: an $(\alpha\beta)_2$ -heterodimer; α denotes a 120 kD protomer consisting of 7 membrane spanning helices. The domains extending into the aqueous phases contain the binding site for the promoter ATP at one side and that for regulating steroids at the opposite one. The α -dimer is supposed to form the channel. The β -domain denotes the associated glycoprotein with molecular weight of 40 kD. It consists of about four membrane spanning helices and is supposed primarily to maintain the correct orientation of the protein within the bilayer.

membrane proteins with a molecular weight of 400 kDalton and their structure is not well known. The H^+ -translocation is powered by ATP, but in contrast to the P-type ATPases, the process does not require a covalent linkage of the phosphate group to the protein associated with a conformational change of the protein (see fig. 12).

Pumps driven by chemical potential gradients: co-transporters and antiporters

This completely different type of pump transports ions (K^+ , H^+ , Ca^{++}) or molecules (sugars required as nutrition) against their respective concentration gradient by simultaneous translocation of a driver ion (in general Na^+) in the direction of its electrochemical gradient. The two species may flow in the same (co-transporters) or in opposite directions (anti-porters). An example of the latter type is the Na^+ - Ca^{++} -transporter which helps to establish the low intracellular Ca^{++} -level. In order to establish electroneutrality two Na^+ -ions have to be translocated per Ca^{++} .

Another important example is the Na^+ - H^+ antiporter which plays an essential role for the removal of excess protons generated in the cells during metabolism. The Na^+ -driven transport also plays an essential role for the import of sugar molecules into cells. Thus, glucose and amino acids are transported from the lumen of the intestine into the blood stream through both membranes of the epithelial cell layer. It is also the primary transport mechanism of glucose or galactose into bacterial cells.

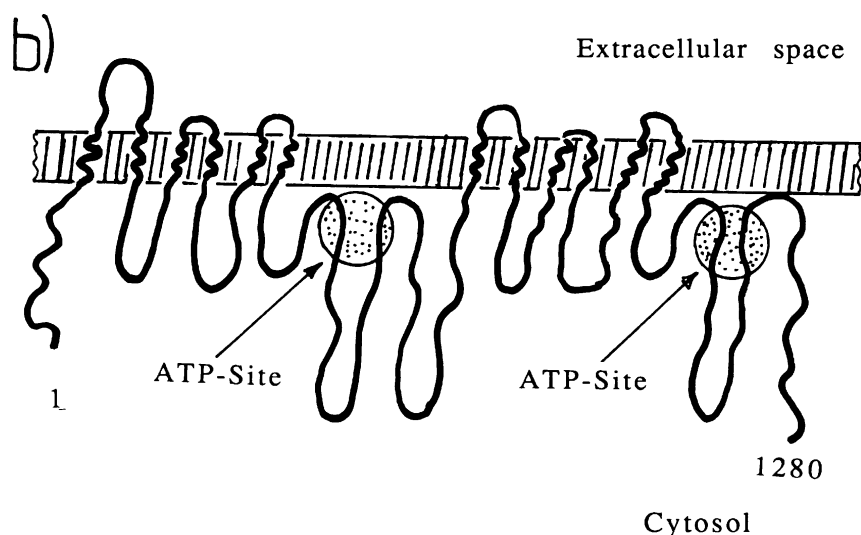


Fig. 11b. Model of multidrug transporters which are most abundant in cell plasma membranes of cancer cells. The translocation of a large variety of drugs is ATP-dependent and the ATP-binding sites are located at cytoplasmatic domains.

Light driven charge pumps

These were most probably the first pumps appearing in evolution. They comprise the H^+ and Cl^- pumps of halobacteria and the reaction centers of photosynthetic bacteria and of chloroplasts. The latter are essentially electron-proton-pumps (Photon-ases).

Multi drug transporters

The recent discovery of this type of pump is a most exciting new development and an essential step towards the understanding of the action of drugs. This 170 kDalton glycoprotein shown in fig. 11b has been discovered in large quantities in tumor cells. They can transport a large class of drugs from the cytosol to the extracellular space. It is thus most probably responsible for the fact that cancer cells can become resistant to chemotherapeutic treatment.

4.2. P-type ATPase is a prototype of a functional protein which is powered by ATP-cleavage and driven by a conformational change following phosphorylation

The ion translocation by P-type ATP-ases is one of the countless biological processes powered by ATP and triggered by phosphorylation of the protein followed by a conformational change of the enzyme. Figure 12 shows the present understanding of the process which occurs in several stages. It can be described in terms of a two-state model as follows (cf. also [2], chapter 17 or [3], chapter 6):

- the P-type pump possesses two stable conformations E_1 and E_2 ;

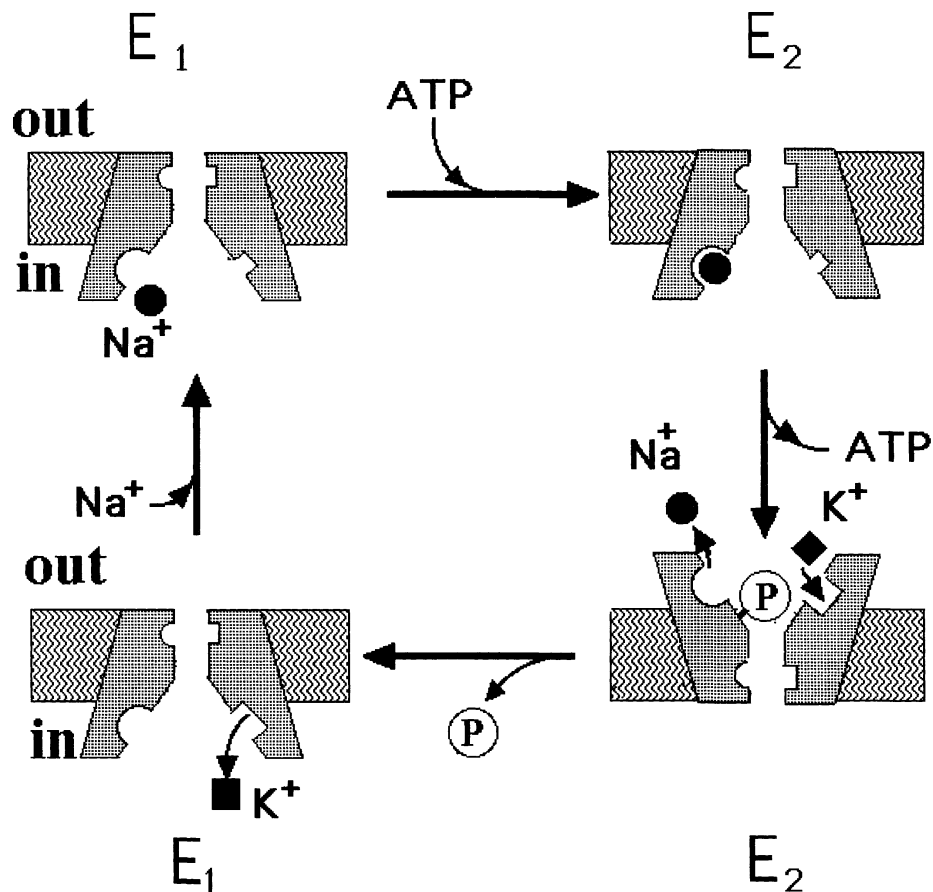


Fig. 12. Model of mechanism of Na^+ - K^+ -antitransport by P-type ATPases. E_1 and E_2 are two conformational states of the protein and the $E_1 \rightarrow E_2$ transition is triggered by phosphorylation. The uptake of Na^+ and detachment of K^+ at one side is due to the high (low) affinity of E_1 for Na^+ (for K^+) and the reverse behaviour for the E_2 -conformation. The $E_1 \rightarrow E_2$ transformation is associated with the turn-over of both binding sites on the protein from one side of the membrane to the other.

- the former is stabilized by phosphorylation (which requires Mg^{++}), the latter by release of the phosphate group P_i . E_1 has a high affinity for Na^+ and a low for K^+ while E_2 behaves in the opposite way;
- Na^+ -binding activates the phosphorylation and thus triggers the $E_1 \rightarrow E_2$ transition while K^+ triggers the P_i detachment and thus the $E_2 \rightarrow E_1$ process;
- the conformational transition $E_1 \rightarrow E_2$ translocates the binding sites for both K^+ and Na^+ from one side of the membrane to the other.

The pump process consists in a cyclic sequence of the following events:

Binding of Na^+ at one side of the membrane triggers the coupling of ATP to an aspartate group. Hydrolysis of ATP induces the $E_1 \rightarrow E_2$ transition by which the ion binding sites are translocated to the other side of the membrane. There Na^+ is

released owing to its low affinity for the E_2 -conformer while K^+ is simultaneously adsorbed. The phosphate release induced by K^+ -binding triggers the $E_1 \rightarrow E_2$ conformational change by which both binding sites are translocated to the original side. K^+ is released from the dephosphorylated E_1 -state and the cycle is completed.

The Ca^{++} -ATPase works in a closely analogous manner. In this case Ca^{++} has a very high affinity for E_2 with a dissociation constant of $K_D = 10^{-7}$ M but a low affinity for E_1 with $K_D = 10^{-2}$ M. It is this large difference which enables the maintenance of the cytosolic Ca^{++} content below the μ M level.

4.3. Energetics and reversibility of pumps

The membrane translocators are reasonably well optimized with respect to the energy source chosen. Let us consider the case of the Na^+ -ATPase. The Na^+ -concentration is 12 mM inside and 145 mM outside of typical mammalian cells. This corresponds to a free energy of

$$\Delta G = RT \ln \frac{[Na^+]_{in}}{[Na^+]_{out}} = -5.8 \text{ kJ} \times M^{-1}. \quad (1)$$

To this we must add the electrochemical potential required to translocate Na^+ against a membrane potential V_m . For $V_m = -70$ mV, which is typical for a mammalian cells, this corresponds to $\Delta G_{el} \approx -12$ kJ/Mol. The total energy stored in one phosphate bond in ATP is $\Delta G = 30$ kJ/Mol which is more than sufficient to drive the Na^+ -translocation.

In the case of the Ca^{++}/Na^+ antiporter the energy required to pump Ca^{++} from a 0.1μ M to a 1 mM concentration at a membrane potential of $V_m = -70$ mV would be

$$\Delta G = -RT \ln \frac{[Ca^{++}]_{in}}{[Ca^{++}]_{out}} - 2FV_m = -35 \text{ kJ} \times M^{-1} \quad (2)$$

where F is the Faraday number. Such a gradient could only be maintained by a Ca^{++} -ATPase in combination with an activator such as calmodulin ([1], chapter 14). The energy stored in the Na^+ -gradient and estimated in eq. (1) would just be sufficient to maintain a concentration ratio of 100 since two Na^+ are translocated for each Ca^{++} .

A remarkable property of transport proteins is their reversibility. The most prominent example is the ATP-synthetase (a F-type ATPase) which powers the formation of ATP by exploiting pH-gradients as energy sources. In the presence of excess ATP it may, however, function as proton pump under ATP-cleavage. For this reason, it is often called an H^+ -ATPase.

4.4. The regulation of Na^+/K^+ -ATPase activity by steroids as a prototype of drug action

The modulation of the activity of P-type pumps by steroids is one of the few cases where drug effects can be understood on the molecular level. The binding of digitalis

(a steroid drug activating the heart muscle) to the appropriate binding side located at the extracellular side of the (heart) muscle cell inhibits the pump (compare fig. 11). This leads to an increase in the intracellular Ca^{++} -level which promotes the muscle activity already at a concentration of 10^{-8} M of digitalis.

4.5. *Differentiation is often controlled by type and number of membrane associated pumps*

The differentiation, e.g., the formation of specialized cells and intracellular compartments is to some extent accomplished by genetic control of the relative number of molecule- and ion-transporters. Thus nerve cells contain a high density of Na^+/K^+ -ATPases while erythrocytes carry only a few of these species. The sarcoplasmic reticulum of muscle cells are distinguished from the endoplasmic reticulum of the same cells by a high number of $\text{Ca}^{++}/\text{Na}^+$ -ATPases. Lysosomes obtain their characteristic high degree of acidity by incorporation of a high content of V-type ATPases into the membrane.

4.6. *The concerted action of many pumps holds electrochemical equilibrium and may control cell shape*

One universal task of membrane bound pumps is the control of osmotic equilibrium. In the most thoroughly studied example, the erythrocyte, the control is performed by four major proteins, possibly in combination with the phospholipid translocase: the Na^+/K^+ -ATPase, the Ca^{++} -ATPase, the Na^+/K^+ -antiporter and the band III anion exchanger. Osmoequilibrium is further determined by the concentrations of impermeable macro ions; such as the hemoglobin and polyphosphate in the cytosol and glucagene in the blood plasma. Under physiological conditions the intracellular pH and osmolarities are maintained at pH 7.4 and 150 mosm. The membrane potential is $V_m = -6$ mV with the cytosol being negative. The fluids are mainly buffered by the $\text{CO}_2/\text{HCO}_3^-$ -system and by hemoglobin (Hb). The CO_2 -transport into the blood plasma in the form of HCO_3^- is mediated by Band III via $\text{HCO}_3^-/\text{Cl}^-$ exchange. The electrical potential is determined by the inside-outside distribution of the major ions, K^+ , Na^+ , Cl^- , Ca^{++} the values of which are given in fig. 13a.

The cells respond to perturbations (i) of the osmotic equilibrium and (ii) of the ion composition at constant osmolarities in a graded manner [11]:

The first response (stage A) is a rapid re-establishment of the osmotic equilibrium by water exchange which occurs with a response time below 1 second. The second stage (stage B) is the equilibration of the chemical potentials of the Cl^- -ions and of the pH with a response time of $\leq 10^{+3}$ sec, depending on various conditions. The third step (stage C) is the re-establishment of the specific chemical potential equilibria of the major cations K^+ and Na^+ . This is a very slow process with a response time of the order of 10^{+5} sec. Since the times between the three stages differ by orders of magnitude, the stages A, B and C may be considered as transient thermodynamic equilibrium-states.

The strategy, to respond to the external perturbations or changes in the external milieu by step-wise variations of the different species (water, H^+ , Na^+ , K^+ , Cl^-)

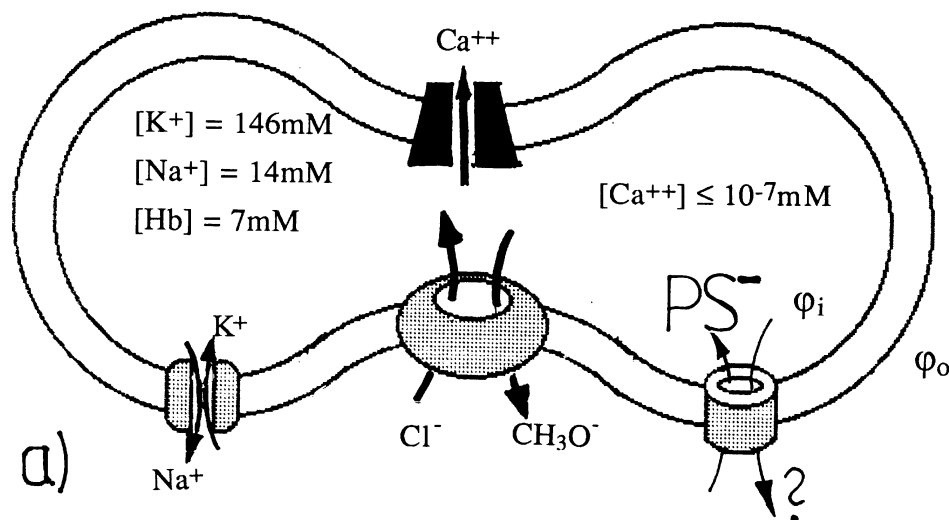
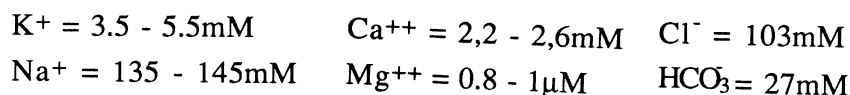


Fig. 13a. Equilibrium concentrations of major ions in the cytosol of erythrocytes and in blood plasma in mM. The membrane potential is $V_m = -6$ mV with the inside negative.

allows the cell to overcome the most dangerous situations such as lysis by lateral tensions or pH-induced conformational changes of the proteins. In particular the rapid water exchange is essential for the survival of the cell.

The graded regulation of the osmotic equilibrium has many practical consequences for the storage of red blood cells or studies of their physical (e.g., rheological) properties. Let us consider two examples:

(1) If the Na^+ -concentration of the external medium is changed at constant osmolarity (e.g., by replacing part of NaCl by saccharose) the cells are slightly deflated even after re-establishment of the cation equilibrium.

(2) Time is an essential physical parameter. Not only the lifetime of a cell but also the time elapsed between a perturbation (e.g., a change of the external medium at constant tonicity) and observation has to be considered for the interpretation of experimental results. An example is the drastic change of membrane voltage, V_m , at the transition from the stage B, i.e. after Cl^- and pH equilibration to the final equilibrium, i.e. stage C. At external Na^+ -concentrations < 100 mM, the membrane voltage is positive in stage B and negative in stage C, (although smaller than under physiological conditions of 140 mM external Na^+ -concentration).

Erythrocytes are also the most prominent examples of shape transitions of cells and vesicles induced by volume changes, osmotic effects or variations in the membrane asymmetry (spontaneous curvature). Some examples are shown in fig. 14. The most essential results are:

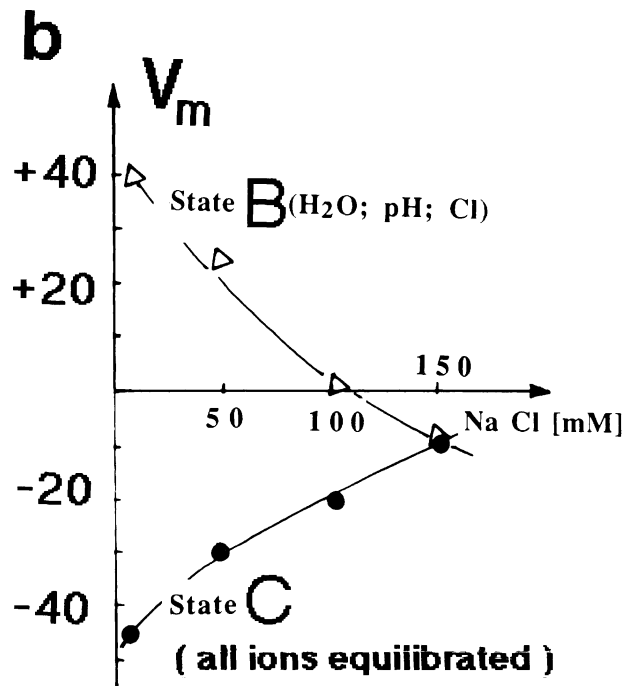


Fig. 13b. Variation of membrane potential $V_m = \varphi^{in} - \varphi^{out}$ with NaCl content of an isotonic solution of saccharose for the intermediate state B and the final state C of the cell. The tonicity ($[Na^+] + [Cl^-] + [saccharose] = 300$ mM) corresponds to physiological conditions. Note that the physiological NaCl concentration is 150 mM. Note that B is the quasi-static state where the osmotic pressure, the pH and the Cl^- -concentration are equilibrated and C the state where all ions are equilibrated after a change in the external medium.

- the shape transitions exhibit typical features of phase transitions, that is they are reversible and may occur continuously (2nd order-like transition) and discontinuously (1st order like).
- they can be induced by variations of the area-to-volume ratio of the cell or changes in the asymmetry of the composite membrane (e.g., relative area changes between the lipid/protein bilayer and the spectrin/actin network)
- shape transitions may be (but need not be) associated with lateral inhomogeneities of the membrane. An example of the former (homogeneous type) is the discocyte-stomatocyte transition and of the latter the discocyte-echinocyte transition.

As will be shown in chapter 8 of this volume, these shape changes may be explained in terms of a very simple concept: the minimum bending energy principle. One unique feature of erythrocytes is that the shape changes may be controlled both by osmotic variations of the area-to-volume ratio of the cell and by changing the membrane asymmetry. Thus, addition of PS to the outer membrane leaflet leads to echinocyte formation. However, the cell re-transforms to discocytes within minutes

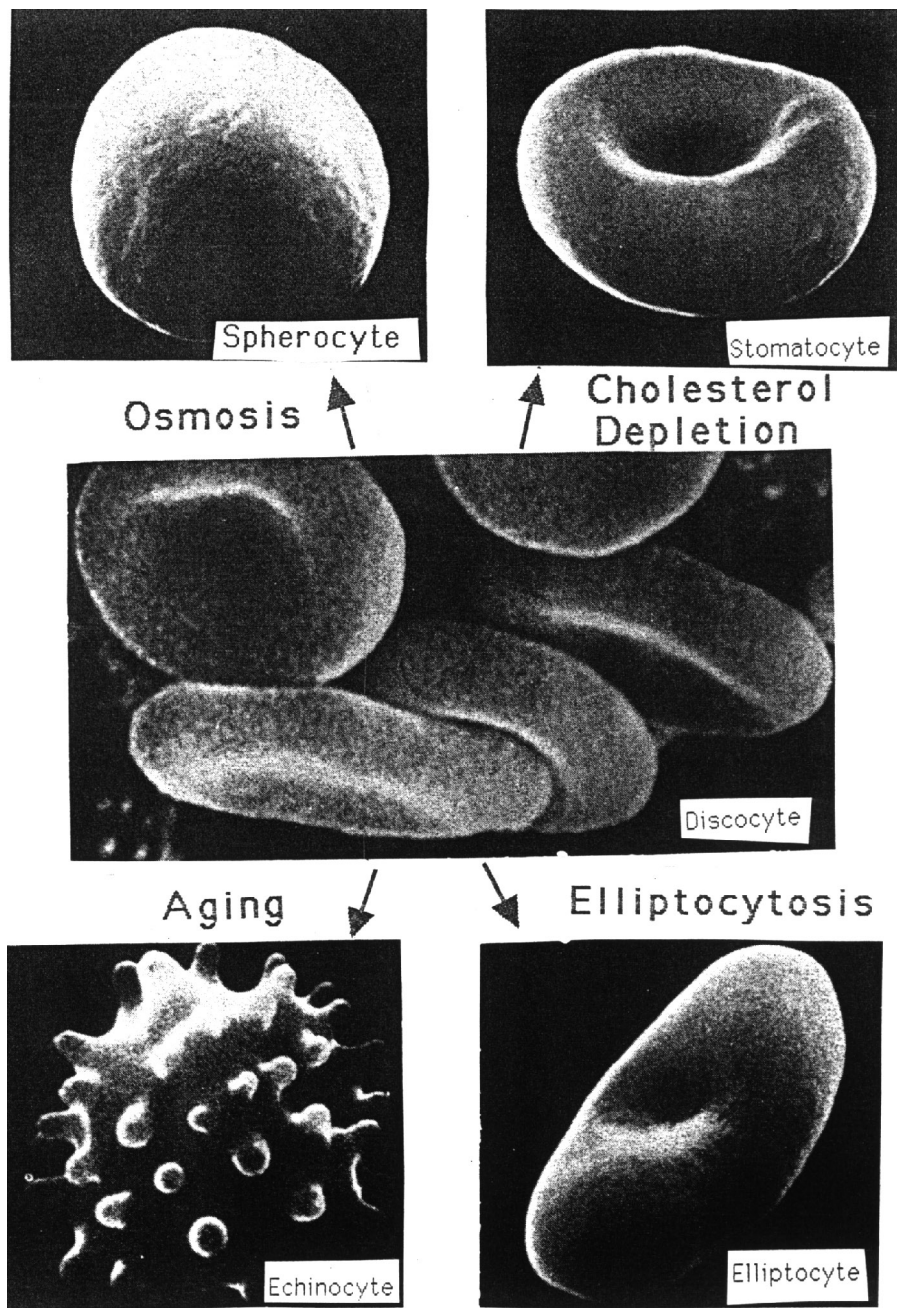


Fig. 14. Various shapes of erythrocytes. Transition between the various shapes can be triggered biochemically by metabolic defects or diseases. The discocyte in the center represents the resting state.

most probably due to the transfer of the charged lipid to the inner leaflet by flippases. This is a beautiful example for the finetuning of cellular shapes.

5. Membrane synthesis, differentiation and recycling

One central and fascinating question is how cells maintain the unique lipid and protein composition of their organelles and how the synthesis of the right quantities of individual proteins and lipids is controlled. Another open question is whether there exists a concerted synthesis of lipids and proteins mediated by selective lipid-protein interaction mechanisms?

Most of the lipids and proteins are synthesized in the endoplasmatic reticulum from where they are transported to the various organelles either in a concerted way mediated by vesicles or as individual entities. Over the last few years the questions of sorting and trafficking of membrane constituents have come into focus in membrane research and much experimental data have been accumulated. In the following the essential principles of membrane synthesis, differentiation, trafficking and recycling are summerized. An excellent and easily understandable introduction can be found in reference [1], chapter 17.

5.1. Lipids are synthesized in the ER, the Golgi and the Mitochondria

Phospholipids are synthesized in the outer monolayer of the ER membrane from one precursor: diacylglycerol [19].

The phospholipids are assembled from fatty acids and the phospholipid backbone glycerol-3-phosphate by successive transfer of the former to the latter constituent (cf. references [17, 19]). This process is mediated in the outer monolayer of the ER membrane by a fatty acid binding protein: the coenzyme A. First phosphatidic acid is made. From this the phosphate group is removed again (by phospholipase) and the resulting (rather hydrophobic) diacylglycerol is thus the real precursor for all the other classes of phospholipids. The various head groups (choline, ethanolamine, serine) are attached to the diacylglycerol precursor by group specific enzymes (so called transferases). The same precursor is used for the synthesis of cardiolipin which occurs in the mitochondria via the intermediate lipid phosphatidylglycerol.

Sphingomyelins are assembled in the monolayer of the luminal side of the Golgi complex by transfer of phosphatidylcholine to ceramides, while the latter are synthesized in the ER.

The more than 15 types of gangliosides are synthesized in the ER and in the Golgi membrane. The precursor is ceramid to which the sugar molecules are attached, one at a time, by specific enzymes. These so-called glycosyltransferases are all integral membrane proteins. This process occurs in the monolayer facing the lumina, and the sugar residues are imported into these spaces from the cytosol (in an activated form with attached nucleotides (cf. reference [1], chapter 17).

5.2. Membrane and secretory proteins are synthesized by the ER and modified in the Golgi: a first step sorting

Both membrane proteins and secretory proteins to be stored in the lumina (such as insulin) are synthesized by the ribosomes attached to the ER membrane while species required in the cytosol (such as spectrin and cytosolic enzymes) are created at freely dissolved ribosomes. The integral proteins are inserted into the membrane during the process of synthesis. Similarly, the nascent secretory proteins are translocated through the membrane into the lumen of the ER. In this way a first step of protein sorting is achieved, since each class of protein resides already in the appropriate subspace.

The integral and the secretory proteins are both transferred to their proper space (the ER membrane or its lumen) by the same complex transfer system shown in fig. 15. It consists (1) of a membrane bound receptor (called SSR = signal sequence

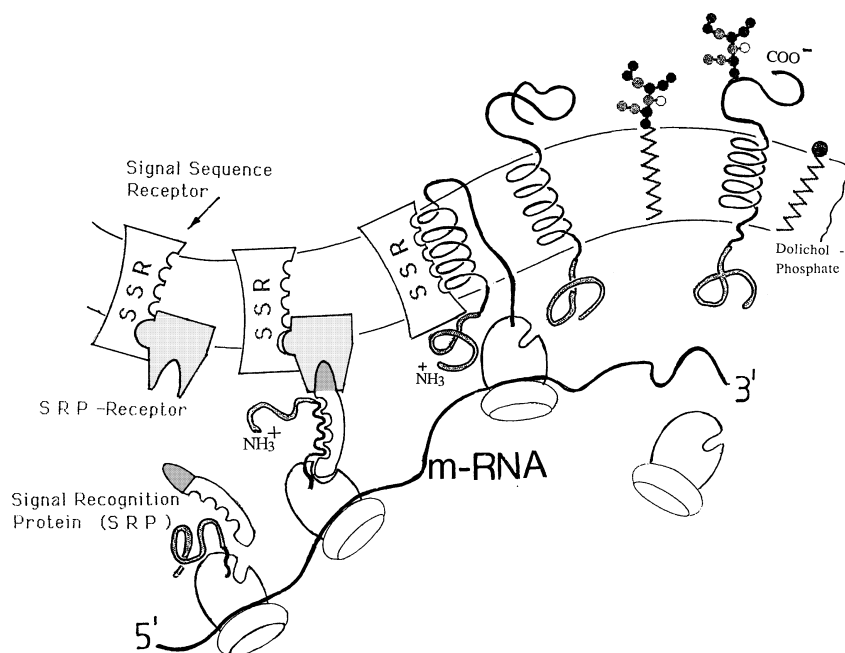


Fig. 15. Model of synthesis of a membrane receptor (a branched polypeptide-oligosaccharide copolymer) and the simultaneous incorporation of a nascent protein into the bilayer. The major trick is the attachment of a (universal) signal sequence to the nascent polypeptide chain which is recognized by the translocation machinery. The latter consists of (i) a receptor (SSR) for the signal sequence which is embedded in the ER bilayer, (ii) a protein-RNA-complex (called signal recognition protein = SRP) which recognizes both the signal sequence and the initial sequence of the protein and (iii) a receptor for the SRP which mediates its coupling (with attached nascent protein) to the (membrane integrated) signal sequence receptor (SSR). After attachment of the loaded SRP to the membrane receptor, the SRP and its receptor are released and the signal sequence couples to its receptor in such a way that the (first) hydrophobic domain of the growing chain penetrates into the bilayer. In the case of secretory proteins which are initially translocated into the ER-lumen, the signal sequence is cleaved off the protein after penetration of the NH_3^+ -end of the nascent protein into the lumen. On the right side is shown the transfer of oligosaccharide from dolichol to newly synthesized receptor. The sugar moiety is further modified in the Golgi-apparatus.

receptor) recognizing a specific amino acid sequence (also called signal sequence) at the leading edges of the nascent proteins; (2) a protein-RNA-complex (called signal recognition protein = SRP) which recognizes the 'signal sequence' when it is still associated with the ribosome and (3) a receptor for the SRP-complex which mediates the coupling of the signal sequence to the signal sequence receptor (SSR). The key element of the transfer system is the 'signal sequence' of the nascent protein which may either precede the proper protein sequence or may be part of the protein as in the case of fig. 15.

The presently accepted view of the synthesis and translocation of the nascent membrane proteins is illustrated in fig. 15 for the case of a membrane receptor. The synthesis is started from the NH₂-end. The emerging amino acid sequence consists of two parts: the cytosolic domain and the hydrophobic domain which is to become the α -helical anchor. The hydrophobic sequence is recognized by one end of the SRP. The leading end of the SRP with the attached protein sequence is recognized and bound by the SRP-receptor. The SRP/SRP-receptor complex is bound to the signal sequence receptor (SSR). This step is followed by the penetration of the hydrophobic sequence (the final membrane spanning part) into the bilayer to such an extent that the initial part of the hydrophilic N-terminus of the protein sticks out into the ER lumen. Simultaneously the SRP/SRP-receptor complex is set free. Finally the hydrophilic part penetrates and the protein detaches from the signal sequence receptor.

The same procedure is supposed to hold for the concerted synthesis and translocation of membrane proteins with several α -helices meandering through the lipid bilayer and for the secretory proteins (cf. reference [1], chapter 17, figs 17–18).

It should be realized that hydrophobic or amphiphilic polypeptides could also penetrate spontaneously into bilayers, driven by the hydrophobic effect. It is hard to imagine that such a process could be controlled by genetic expressions.

5.3. *Nascent proteins must be processed in various ways before their translocation to target spaces and membranes*

Only proteins which are properly folded and carry the proper oligosaccharides can be secreted or transferred from the ER to the target membranes (e.g., by vesicles into the Golgi). Incompletely folded proteins remain in the lumen of the ER and are degraded as is the case for proteins modified by mutations. This so-called post-translational processing involves

- The formation of disulfide (–Cys–S–S–Cys) bonds.
- The assembly of the various polypeptide chains of a protein oligomer such as the two heavy and the two light chains of an antibody.
- The attachment of the proper oligosaccharides (glycosilation).

The former two conditioning processes appear to be located solely in the lumen of the ER while oligosaccharides are attached to proteins and lipids in the lumina of both the ER and the Golgi apparatus. One distinguishes between two types of linkages: (1) so called N-linkages to the NH₂-group of the amino acid asparagin and (2) the O-linkage to OH-side groups of amino acids:

- The N-linkage occurs in the ER and is used for the attachment of large pre-synthesized oligosaccharides. It is accomplished by their transfer from the

membrane bound dolichol head group to the protein domain extending into the lumen of the endoplasmic reticulum. Figure 15 shows a schematic view of the transferase mediated process.

- By the O-linkage, short sugar oligomers are in general attached (with the notable exception of blood group antigens coupling to galactosides). The O-linkage occurs (as in the case of the ganglioside synthesis) by subsequent transfer of the sugar molecules (one at a time). This type of linkage can occur in the lumen of both the ER and the Golgi and is again catalysed by membrane residing transferases.

Many glycoproteins and glycolipids are conditioned in the Golgi by modification of the oligosaccharide. This consists often of the replacement of glucose by other sugar groups. It is catalysed by enzymes and occurs in a sequential manner in different Golgi compartments. Most interestingly, the modifications happen often only a few minutes before the molecules appear in the plasma membrane or are secreted.

5.4. Part of newly synthesized lipid and protein molecules are distributed on a molecular basis

Most of the newly synthesized lipid is transferred to the target organelles via vesicles. However, there is also the possibility of molecular transport mediated by a whole phalanx of specific phospholipid exchange proteins. As is well known, these transporters exchange phospholipids between vesicles of different composition very effectively. They may effect for instance the transport of PE and PC from the ER to the outer mitochondrial membrane.

Newly synthesized globular proteins for organelles such as mitochondria and chloroplasts (which are not coupled to the normal pathway of insertion of newly synthesized membrane via vesicle trafficking) are transported into these organelles by specific transport proteins. This holds also for most proteins required for oxidative phosphorylation in the mitochondria which are synthesized at free cytoplasmatic ribosomes and are subsequently translocated through the two membranes.

As illustrated in fig. 16, a trick similar to that in the translocation of integral and secretory proteins through the ER membrane is used (cf. reference [1], chapter 18 for details). The first step is the unfolding of the enzymatic moiety of the protein. This process is mediated by a class of proteins called chaperons and requires ATP. So-called heat shock proteins, which bind to thermally denaturated proteins and prevent their precipitation in cells, also belong to this class. The signal sequence of the unfolded protein is recognized and bound by a membrane-bound receptor. It diffuses to a site of tight contact between inner and outer membrane of the mitochondrion where it is translocated across both membranes into the matrix (compare references [20] and [1], chapter 18 for details). This is supposed to occur through a channel. Within the matrix the targeting sequence is cleaved and the enzyme moiety folds into the active conformation. Often this protein re-folding step requires helper proteins (such as chaperons).

More complex is the transfer of proteins (such as cytochrome C₁ of the electron transfer chain) which have to be targeted to the inter-membrane space of the mitochondria. These proteins contain two targeting sequences. The first helps to direct

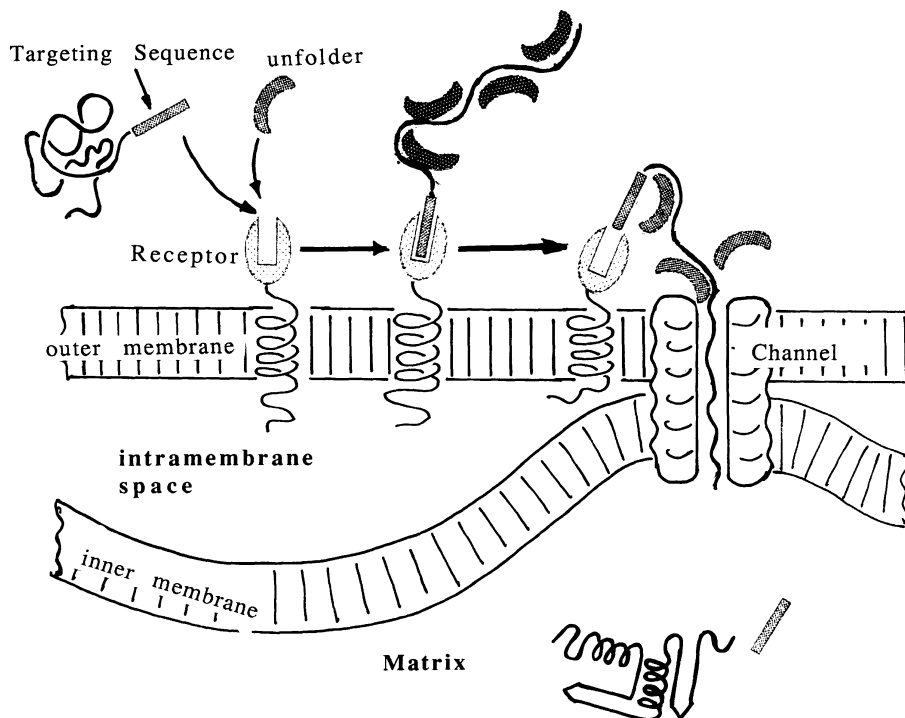


Fig. 16. Model of transport of proteins synthesized by cytosolic ribosomes into mitochondria (post-translational import). The polypeptide consists of the enzyme moiety and a signal sequence. First step (ATP-driven): unfolding of enzyme moiety by binding of unfolding proteins called chaperons (analogues of heat shock proteins and by recognition of signal sequence by membrane bound receptors. Second step: translocation of unfolded protein through channel spanning both the inner and outer membrane at contact site. Mitochondria contain 100–1000 such channels. Third step: cleavage of signal sequence (which is then degraded) and refolding of enzymatic moiety into functional conformation. Note also (1) that: proteins can also be membrane spanning; (2) that proteins which are located in inter-membrane space are transferred by a second translocation protein. (Sources: N. Pfanner F. U. Hartl and W. Neupert, *Eur. J. Biochem.* **175**, 205–212 (1988); Darnell et al. [1], ch. 18.)

the protein into the matrix space (and is cleaved there) while the second helps to direct the subsequent transfer through the inner membrane into the inter-membrane space. Evidence for the latter step is provided by the finding that cytochrome b₂, which lack the second targeting sequence, are imported into the matrix but not into the intermembrane space.

The use of signal sequences for the translocation of nascent proteins through the ER membrane and of proteins synthesized in the cytosol through the mitochondrial membranes is another impressive example for nature's strategy to use universal mechanisms for many similar cellular processes. This is further emphasized by the fact that bacteria use the same type of mechanism to transport proteins into the outer membrane [21].

The targeting sequence of mitochondrial proteins are rich in positively charged

side groups (arginine, lysine) but devoid of negative groups and thus exhibit a high positive charge. This opens the possibility for electrically driven protein translocation. Thus, the protein translocation through the two mitochondria membranes is driven by the very large trans-membrane electric potential of about 200 mV. The matrix space is negative thus facilitating the pulling in of the positively charged targeting sequence. This follows from the finding that suppression of the potential by inhibition of the oxidative phosphorylation abolishes the protein translocation. An electric driving force is also essential for the protein translocation in bacteria (e.g., *E. coli*). In this case it is the protonmotive force. The translocation is initiated by ATP-driven binding of the unfoldase protein, while most of the further steps are driven by the protonmotive gradient.

6. Vectorial organisation of cells and material flow during metabolism and secretion

6.1. Synopsis

Order in cells is partially maintained (1) by a vectorial (polar) organisation of the organelles in the cell in the radial direction (from nucleus to plasma membrane) and (2) by a continuous and bi-directional flow of newly synthesized material from the ER to the plasma membrane and of metabolites in the opposite direction. The material exchange serves two purposes: firstly, the import of metabolites and the disposal of waste, and secondly, a continuous renewal of the cell plasma membrane and other cellular organelles. In fact it is estimated that epithelial cells internalize and recycle about 50% of the plasma membrane each hour [1]. Owing to a continuous recycling of a large fraction of the membrane material this trafficking is highly economical. In the following the present view of some essential features of the material flow in cells is summarized. For more details the reader is referred to the excellent presentation by Darnell et al. ([1], chapter 17).

We will see again that these complex and rather different processes are governed by only a few general principles. In fig. 17 the most essential routes of material exchange are summarized for the well studied case of secretory cells. The pathways and principles are similar in epithelial cells, for instance in the kidney. However, in this case the material has to be selectively distributed in two directions: towards the basolateral plasma membrane contacting the blood stream and the apical plasma membrane coupled to the lumen.

Another essential polarity in the cell is established by the different acidities of the lumina of the organelles. The lumina of the nucleus, the ER and the Golgi apparatus exhibit a neutral pH (pH 7–7.3). Lysosomes, however, are strongly acidic (pH 4.5–5) and the endosomes exhibit a pH of 5–5.2. The acidic milieu of lysosomes and endosomes play an important role for the release of metabolites from receptors (e.g., of Fe^{+++} from transferrin) or the conditioning of lysosomal enzymes (proteases).

6.2. Phenomenology of trafficking from the ER to the plasma membrane

Transport is effected on a molecular and a mesoscopic level. The former holds in general for small molecules (e.g., sugar metabolites and amino acids) and ions

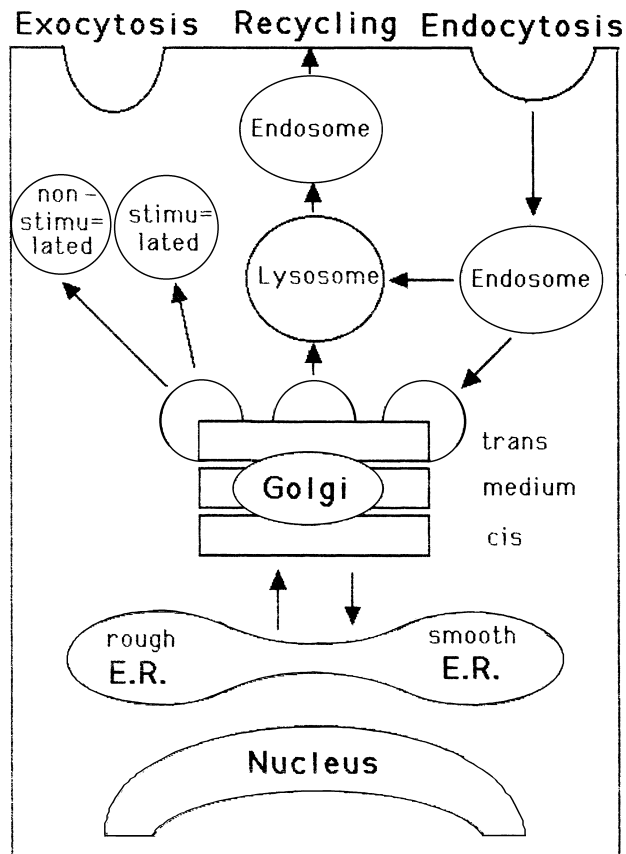


Fig. 17. Vectorial organization of cell organelles and major route of directed material exchange. Newly synthesized membrane and secretory proteins are transported in a sequential manner from the ER over the cis- and medium-fraction to the trans-fraction of the Golgi complex. From this final stage of the sorting and conditioning machinery, the vesicle mediated transport branches into two major routes: (1) the two pathways of (hormone-induced and non-stimulated) exocytosis (left side) and the bidirectional flow between the trans-Golgi and the plasma membrane (right side) which is essentially coupled by the lysosome-endosome vesicular system. The unidirectional flow of secretory products also permits the insertion of new membrane into the plasma membrane to compensate for losses owing to endocytosis and pinocytosis.

and is mediated by pumps and co-transporters. The second route occurs via vesicles and is associated with local membrane instabilities in the course of the vesicle budding→fission→fusion chain of events. This latter route of traffic will be considered below in more detail.

Vesicle exchange is governed by conservation of the total membrane area of the various organelles (at least within certain limits). This is achieved by continuous recycling of membranes. Consider for example the plasma membrane of secretory cells. The fusion of vesicles with the plasma membrane during exocytosis would imply a continuous expansion of the cell envelope. It is thus counterbalanced by

internalization of vesicles by endocytosis. Since this process is a major route of uptake of metabolites such as low density lipoproteins or iron (mediated by transferrin), material transport through the cell envelope and preservation of the membrane area can be controlled in a most economical way.

Consider now the vesicle flow between the ER and the plasma membrane in some detail. Newly synthesized membrane together with secretory proteins are transported in a sequential manner (via vesicles) from the (rough and smooth) ER over the cis-fraction, and the medium fraction to the trans-moiety of the Golgi complex. The latter compartment is the final stage of a sorting machinery from where the vesicular transport branches into two major pathways: the (two) routes of exocytosis of secretorial proteins and the lysosome/endosome vesicle system. The latter mediates the bidirectional flow between the plasma membrane and the trans-fraction of the Golgi apparatus.

The first step, the vesicular transport from the ER to the cis-Golgi is demonstrated by the fact that the intermediate space is filled with an accumulation of vesicles which have been pinched-off from protrusions emanating from the ER membrane as is demonstrated in fig. 18a.

The next step, the transfer from the cis to the medium Golgi is mediated by coated vesicles as has been demonstrated at least in the case of cultured liver cells (cf. [1],

Fig. 18a. Electron micrograph of overall organization of ER, Golgi fractions and lysosomes together with the coupling vesicles. One can clearly distinguish the polar arrangement of the ER and the Golgi complex which are separated by a manifold of vesicles. The formation of a secretory vesicle is also shown at the top (after reference [3]).

Fig. 18b. Electron micrograph demonstrating that transport of material between cis and medium (or trans) Golgi fraction is mediated by coated vesicles. Note the splitting of vesicles with coat (indicated by thick arrows) from cis-Golgi fraction. The protein coat is different from clathrin. (Source: Melancon et al., *Cell* **51**, 1053–1062 (1987), [22].)

figs 17–32 and [22]). The formation of the coat followed by budding of the coated pit and detachment of the vesicles is triggered by ATP. The coated vesicles bind to acceptors of the medium-Golgi membranes. The fusion with the latter occurs in two steps: first, the detachment of the coat from the vesicle, which is mediated by GTP

hydrolysis, and second, fusion, a process requiring fusinogenes (cf. chapter 19). The first step has been verified by the finding that an analogue of GTP which cannot be hydrolysed inhibits uncoating. The fusion itself is mediated by small polypeptides and requires ATP hydrolysis. It is important to note that the coat protein is clearly different from clathrin, as has been verified by immuno assays.

The third step, the transfer of vesicles from the medium to the trans fraction of the Golgi complex is less well known. In particular, it is not known whether it too is mediated by coated vesicles. However, as will be stressed below, the exchange between trans Golgi and endosomes is mediated by clathrin coated vesicles.

Two different types of exocytosis, which may coexist in one cell type such as pancreatic cells are observed: the continuous secretion and the externally triggered exocytosis (induced by hormones, Ca^{++} or antigens). Newly made cell surface receptors are inserted into the plasma membrane by continuous secretion. The stimulated process is used for the secretion of hormones such as insulin in the case of pancreatic cells. Another example is the exocytosis of histamines by mast cells during an allergic reaction.

Both types of secreted proteins are dissolved initially in the same compartment of the trans Golgi. How are these different types of secretory proteins sorted into the right type of transport vesicles? Detailed information is available only for the stimulated process. Several small proteins (≈ 25 kD) have been discovered which bind to the stimulated class of secretory proteins (such as insulin) but not to proteins that are secreted continuously. The binding causes aggregation of the secretory proteins. This aggregation triggers the budding and detachment of secretory vesicles from the trans-Golgi. These contain a high density of aggregated secretory proteins held together by 25 kD proteins, and exhibit a clathrin coat. The newly formed secretory vesicles mature further by a change of the luminal pH from neutral to pH 5.5. This leads to the detachment of the 25 kD binding proteins which are most probably recycled back to the trans-Golgi. In some cases the secretory proteins are simultaneously modified. An example is insulin which is formed at the acidic pH from its precursor proinsulin.

The final exocytotic step is triggered by a transient intracellular Ca^{++} increase which is induced by the hormone binding. This process is reminiscent of the Ca^{++} -induced triggering of fusion of synaptic vesicles with the presynaptic membrane leading to the secretion of neurotransmitters. This is in agreement with the finding that fusion of the mature secretory vesicles with the plasma membrane can be triggered by Ca^{++} -ionophores which increase the intracellular Ca^{++} -level.

A very interesting membrane process to be mentioned in this respect is the fusion of many secretory vesicles in the cytosol into larger vacuoles which then fuses with the plasma membrane, resulting in the simultaneous export of a large amount of proteins. This trick is used by mast cells to attack large enemies like parasites or by reticulocytes to export residual organelles.

6.3. On the generation of lysosomes and lysosomal enzymes

A beautiful example of directed targeting is the generation of lysosomes associated with the stepwise modification of lysosomal proteins. The process is illustrated

in fig. 19. One key step is the 'labelling' of the oligosaccharide head group of the lysosomal enzymes by attachment of one (or several) phosphate group(s) to a mannose residue. One famous 'label' is the mannose-6-phosphate, formed by phosphorylation of mannose residues within the cis-Golgi lumen (cf. [1], chapter 17, fig. 17.35). The mannose-6-phosphate group is recognized by a special receptor in the membrane of the trans-Golgi after transfer of vesicles from the cis- to the trans-Golgi fraction (step 2 in fig. 19).

In the next step, membrane regions within which loaded receptors have been accumulated bud and pinch-off from the trans-Golgi. After fusion of the detached vesicles with a vesicle fraction of low pH (≤ 5) (step 3 in fig. 19), the enzyme dissociates from the receptor and, in order to prevent rebinding, the phosphate groups are cleaved by phosphatases. The final step is the fission of these so called CURL vesicles (see fig. 19 for definition) into a lysosome and a transport vesicle. The latter re-fuses with the trans-Golgi membrane, thus recycling the receptors.

The discovery of the above process of lysosome formation is an interesting example of the exploration of cellular events based on studies of genetic defects. Patients lacking the transferase that mediates the phosphorylation of mannose are deficient of many lysosomal proteins (e.g., in the macrophages or fibroblasts) for the following reason. Since the non-phosphorylated lysosomal proteins are not recognized by the mannose-6-phosphate receptor they are secreted. An accumulation of waste in the cells, results in a lethal toxicity. Other lysosomal proteins or the mannose-6-phosphate receptor are, however, present.

6.4. Receptor mediated endocytosis of metabolites via coated pits: an example of vesicle and receptor recycling

The uptake of iron (Fe^{3+}) by cells is a beautiful example of receptor mediated import of materials by cells combined with recycling of membrane receptors. Membranes are impermeable to Fe^{3+} and its import must be mediated by receptors. The sequence of events is presented in fig. 20. The elements of the import machinery are: (1) the Fe^{3+} binding protein transferrin, (2) the transferrin receptor and (3) the membrane coating protein clathrin with its associated auxiliary proteins (cf. below).

The process begins with the accumulation of loaded receptors in certain membrane areas. This patching is most probably mediated by the simultaneous adsorption of a coat of clathrin molecules to the inner membrane leaflet. This process leads to the formation of an invagination: the coated pit and the coated vesicles eventually pinches-off the plasma membrane. The clathrin coat is released by an ATP-driven process and recycled to the plasma membrane. The naked vesicle, now called endosome, fuses with lysosomal vesicles. The iron is released from the transferrin in the now acidic milieu with pH 5. Eventually the hybrid vesicle (occasionally called CURL = compartment of uncoupling of receptor and ligand) becomes unstable and decays into a fraction containing the iron and one enriched with receptors carrying the iron-free transferrin, called apo-transferrin. The latter fuses with the plasma membrane. The transition into a now neutral milieu leads to the dissociation of the apo-transferrin and the cycle is complete. The cycle time is about 15 minutes and a cell can take up of the order of 10^4 Fe^{3+} -ions per minute.

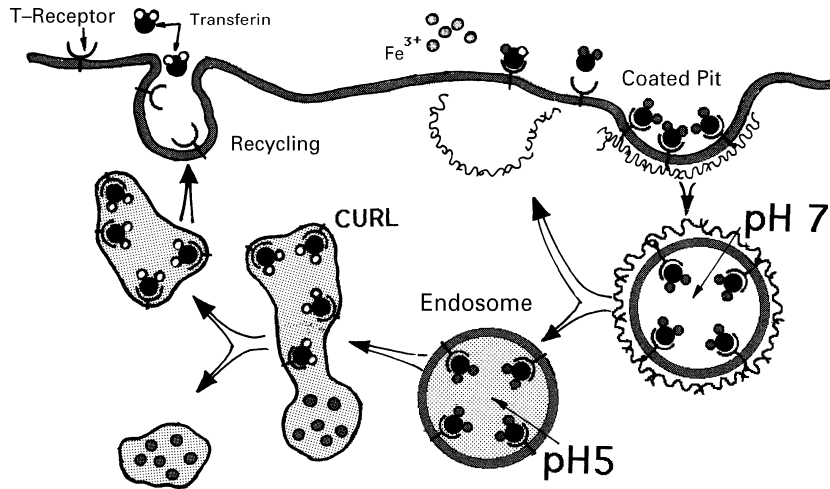


Fig. 20. Model of receptor mediated import of iron and membrane receptor recycling. Iron is bound to transferrin (two ions per ligand) and couples to the transferrin receptor which induces its accumulation in the coated pits. After budding and pinch-off of the coated vesicle the clathrin coat is detached. The naked vesicle (= endosome) fuses with acidic decoupling vesicles (= CURL vesicle or pre-lysosomes), resulting in dissociation of iron. This hybrid vesicle undergoes fission into an iron containing and a receptor enriched part. The latter re-fuses with the plasma membrane and the neutralization of the aqueous milieu causes dissociation of transferrin; thus closing the cycle. The cycle time is about 15 minutes. The dissociation of clathrin from the vesicle is triggered by binding of a cytosolic protein (a relative of the family of heat shock enzymes; cf. review J.F.Rothman and S.L.Schmid, Cell 16, 5-9 (1980)).

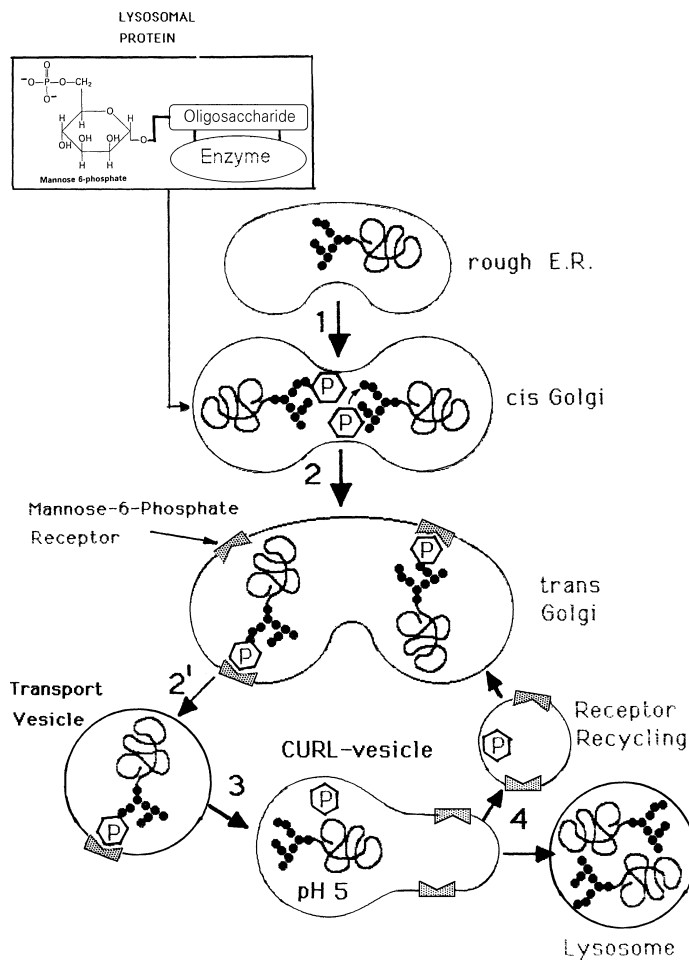


Fig. 19. Example of selective targeting of proteins synthesized in lumen of the rough ER to lysosomes. Step 1 (in cis Golgi): enzymatic phosphorylation of mannose residues of oligosaccharide head groups of protein and transfer to trans-Golgi. Step 2 (in trans Golgi): Binding of phosphorylated oligosaccharide group to mannose-6-phosphate receptors and pinching-off of vesicles carrying mannose phosphate receptors. Step 3: Fusion of vesicle with so called uncoupling and sorting vesicles (sometimes called CURL = compartment of uncoupling of receptor and ligand) and dissociation of enzyme from receptor in pH 5-milieu of CURL. Step 4: Fission of CURL into vesicle containing free mannose-6-phosphate receptor and into free enzyme containing lysosome. Recycling of receptor by fusion of former vesicle with trans Golgi membrane.

The key points are the variation of the pH and ionic milieu during the cyclic process and the pH-dependence of the binding constants: The Fe-transferrin binding constant is high at neutral pH ($K_D = 6 \times 10^{-9}$ M) but so low at acidic pH that it cannot be measured. In contrast, iron-free transferrin binds to the receptor strongly at acidic but weakly at neutral pH. For that reason iron is set free in the acidic milieu of the CURL vesicle and the transferrin in the neutral milieu of the extracellular space.

The iron uptake is a type of receptor mediated endocytosis in which the ligand is recycled. A second type is the LDL import in which the ligand is unfolded and digested in the acidic milieu of the lysosomes while the receptor returns back to the plasma membrane. In a third type, both the receptor and its associated ligand are decomposed in the lysosomes. Examples include the internalization of epidermal growth factors and immuno complexes (cf. [2], chapter 31). A last type of exocytosis which is effective in epithelial cells is the translocation of antibodies residing in the mother's milk to the blood stream of embryos across the epithelial cell monolayer of the latter's intestine (cf. [1], fig. 14.31).

The endocytotic pathway is also exploited by virus to penetrate into host cells.

6.5. *The role of molecular filters for the directed material exchange and sorting*

The astonishingly well-controlled sorting and directed material exchange raises many questions: how is the individual lipid and protein composition of the organelles controlled? How does a vesicle budding from the trans-Golgi know whether it belongs to the lysosomal or secretory fraction?

Several control mechanisms have been discovered recently showing that the sorting of vesicles from the trans-Golgi is regulated by various molecular filtering processes associated with coated pits and coated vesicles.

A new class of clathrin adaptor proteins have been discovered in the 20 nm wide space between the bilayer surface and the clathrin coat: These so called adaptins mediate the accumulation of certain types of receptors within the coated pit [24]. Two complementary species have been found of which one (α -adaptin) is primarily associated with the coated pits in the plasma membrane and the other (γ -adaptin) with the coated trans-Golgi vesicles. Both are widely homologous tetramers composed of two heavy and two light polypeptide chains. One side binds to clathrin while the other exhibits binding sites for the receptor proteins associated with the bilayer membrane.

The genes of several of the receptors that enter the cell by endocytosis (such as the transferrin and the LDL-receptor) have been cloned and their binding to the adaptors has been studied. These experiments together with studies of the modification of the coupling caused by mutations reveal:

- defined sequences (often called motives) comprising 4 to 6 amino acids reside at the cytoplasmatic domain which are recognized by the adaptins
- the binding is not affected by moving these sequences along the chains (by some 30 residues) showing that the recognition does not depend on the configuration of the bulk of the polypeptide chain

- replacement of the recognition sequence of the transferrin receptor by those of other types of receptors does not impair transferrin uptake.

Concerning the recycling of the receptors back to the plasma membrane evidence has been provided that they are passively returned to the plasma membrane via vesicles detached from the CURL-vesicles unless another signal such as phosphorylation directs them to another route such as to lysosomes [24].

The receptors of the trans-Golgi complex (e.g., mannose-6-phosphate receptors) exhibit another recognition sequence for the γ -adaptin. These receptors thereby recycle back to the trans-Golgi fraction after delivery of the lysosomal protein to the lysosomes, cf. fig. 19.

Thus, the lysosomal organelles receive proteins and lipids from two sides: the Golgi and the plasma membrane. It is not yet known whether the two types of receptor mix within the same lysosomal membrane or whether the two pathways are separated. The fact that most of the receptors internalized by endocytosis recycle back to the plasma membrane without mixing with the receptors from the Golgi membrane favors the second alternative.

An interesting finding in this respect is that receptors (e.g., for transferrin) which have been depleted of sialic acids within the carbohydrate groups are internalized but return to the plasma membrane resialyated. This shows that some transfer to the Golgi occurs but that these receptors are eventually retrieved.

The release of clathrin from the coated vesicles is triggered by phosphorylation of clathrin. This process is mediated by an ATPase which is analogous to the heat shock proteins.

There is evidence that the adaptin mediated assembly and disassembly of the receptor with the clathrin coat is also mediated by phosphorylation. This process is in general affected by Ca^{++} -dependent kinases but in some cases also by the receptor itself if it exhibits self-phosphorylation activity such as the insulin receptor.

The adaptin mediated molecular filtering is a highly selective process for protein sorting. But how is the overall lipid-protein composition of the vesicles controlled, for instance during the splitting of the hybrid CURL-vesicle into a lysosome and a plasma membrane fraction (which is then recycled by re-fusion with the plasma membrane)? Here specific lipid/protein interactions together with curvature-induced phase separation and lipid-dependent budding processes could become essential. One possible example is the recycling of receptors by fusion of vesicles detached from lysosomes or CURL-vesicles with the plasma membrane. This process must involve some lipid sorting in order to maintain the characteristic composition of the various membranes. In particular, the sphingomyelins (SPHM) must be effectively recycled to the plasma membrane, since the concentration of this lipid in the Golgi is low as follows from table 2. Interestingly, these lipids contain a high fraction of long chain fatty acids with low degree of unsaturation (e.g., 24% C 24:0 and 23% C 24:1 acyl chains). If vesicles of natural mixtures of SPHM are heated they exhibit lateral phase separation over a temperature range of 20°C to 50°C. As will be shown in chapter 5 this process is associated with vesicle instability leading to budding and concomitant fission of small vesicles containing predominantly the long chain lipids. Thus, if the long chains SPHM's interact predominantly with the receptors, both the receptors and the lipids could be sorted synchronously.

7. On global shape instabilities of cells

The composite structure of cell plasma membranes becomes most dramatically evident if one considers cellular processes associated with global shape changes and chemomechanical processes such as phagocytosis, cell locomotion or cell-cell- and cell-substrate interaction. Some of these processes will be dealt with in detail in chapters 15 and 17. In the following we summarize some essential membrane processes associated with phagocytosis or the expulsion of large aggregates by cells.

7.1. Phagocytosis

Phagocytosis is the engulfment of particles with diameters larger than $1 \mu\text{m}$ by eukaryotic cells, in contrast to endocytosis, by which particles of $< 0.1 \mu\text{m}$ diameter are internalized. Examples of phagocytosis include

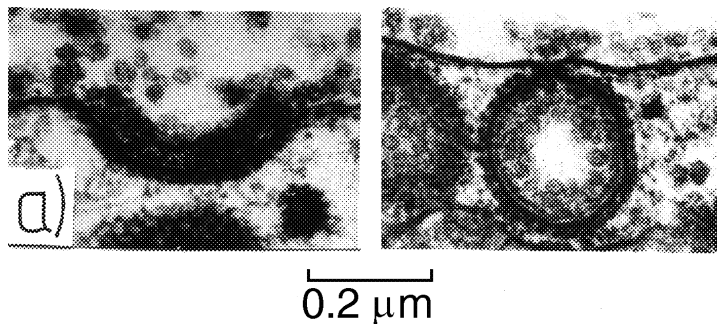


Fig. 21a. Electron micrograph showing one invagination and pinch-off of clathrin coated vesicle.

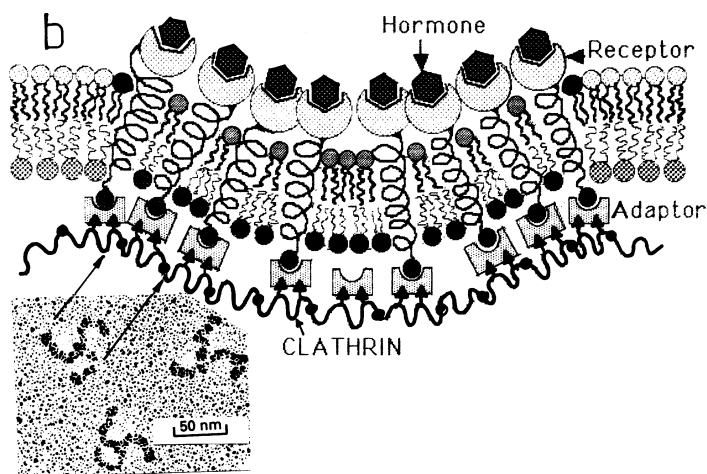


Fig. 21b. Model of coated pit formation by selective binding of clathrin to the receptor via adaptor followed by lateral phase separation. Note that a selective lipid fraction comes with the protein.

Fig. 22. Phagocytosis of red cell ghost by macrophage. Left: initial process of embracement of cell by spreading of macrophage over cell surface reminiscent of a wetting process. Right: Situation after completion of process showing freshly internalized ghost which remains encapsulated in vacuole. The site indicated by arrow shows another ghost at a later state of digestion. (Source: Bessis, *The Red Blood Cell*, Springer-Verlag, Berlin, 1973.)

- the ingestion of nutrition such as bacteria by amoeba (e.g., dictyostelium discoideum)
- the elimination of (1) intruded enemies such as bacteria and viruses, (2) of dying cells as shown in fig. 22 or (3) of inorganic particles such as asbestos from the organism.

The latter tasks are fulfilled in animals by specialized cells called phagocytes. One important class, the macrophages, reside in the narrow channels of the liver or spleen, where they remove aging red blood cells. Others, called, monocytes (or white blood cells) migrate in the body tissue to remove microorganisms such as bacteria.

Phagocytosis exhibits some typical features of wetting processes since it requires a continuous adhesion between the two membrane surfaces. This is demonstrated by the elegant experiments described in fig. 23 showing the engulfment of a lymphocyte. If the receptors (antigens) are uniformly distributed over the whole surface of the victim-cell the plasma membrane of the attacking cell spreads as a thin shell over the whole surface of the lymphocyte, which is finally engulfed. If the receptors on the target cell are clustered together (by capping) the phagocyte spreads only over the region covered by antibodies after which the process stops.

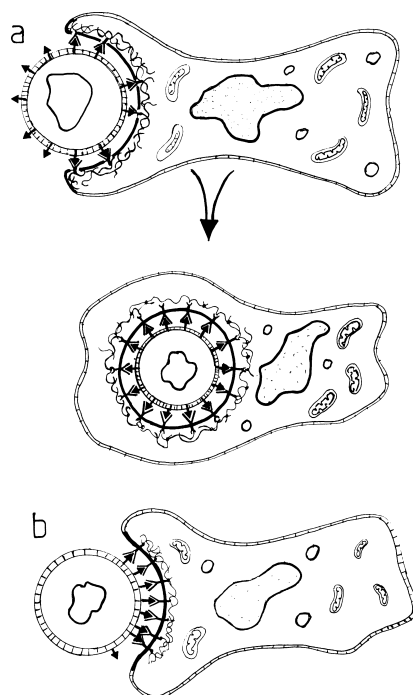


Fig. 23. a) Schematic view of phagocytosis of lymphocyte coated by receptors (in this case immunoglobulin IgG bound to surface antigens) over whole surface. The embracement is actively stabilized by formation of cortex of actin network within lobe spreading over the target cell (often called zipper-process). On the bottom the state after engulfment is shown which is followed by fusion of the internalized vesicle with lysosomes. b) Impedement of engulfment of lymphocyte after aggregation of receptors into patch by so called cap formation (another chemomechanical cellular process). Note that both coupling of antigen to antibody and the actin polymerization are essential for the engulfment.

The first event of phagocytosis is the spreading of a thin layer ($\leq 1 \mu\text{m}$) of the phagocyte over the target cell. This is driven by the coupling of surface receptors of the phagocyte with antigens covering the target cell. (In the case of the removal of lymphocytes by phagocytic leukocytes the former are covered by Y-shaped immunoglobulin (IgG), the stem of which is recognized by the plasma membrane receptors of the latter). When the leading rims of the advancing lobe of the phagocyte meet, the adsorbed and the outer plasma membranes fuse and the target cell together with its bilayer envelope are engulfed. After internalization the vacuole fuses with (primary) lysosomes to form so called secondary lysosomes and the lysosomal proteins such as proteases start to decompose the intruder. Many intruders contain non-digestible materials such as cell walls of microorganisms. These remain in the secondary lysosomes and are one of the causes of the short lifetime of macrophages which is of the order of 1–2 days. This is a remarkable contrast to the life time of 120 days of red blood cells.

The embracement of the target cell by the phagocyte is not a real wetting process. The receptor mediated interaction between the two membranes is most likely only

Fig. 24. Membrane instabilities during expulsion of nucleus and intracellular organelles of maturing erythrocyte. (a) Expulsion of nucleus by budding and fission of vesicle containing the nucleus. The nucleus is surrounded by a membrane and can therefore be engulfed again by the mother cell (cf. fig. 7). (b) Left side: Secretion (merocrine type) of intracellular organelles and decomposition products. First a vacuole is formed by fusion of many vesicles which then fuses with the plasma membrane as in normal secretion. On the right side one can see the temporary pit formed during fusion. (Source: B.Schnitzer, D.Rucknagel and H.Spencer, *Science* **173**, 251 (1971).)

sufficient to trigger the spreading of the lipid/protein bilayer of the phagocyte over the target cell. The actual driving force for the stabilization of the embracement is, however, provided by the simultaneous formation of a thin layer (cortex) of a network of actin filaments above the inner leaflet of the contacting plasma membrane of the phagocyte (cf. fig. 23). Evidence comes from electron microscopic studies by which the actin cortex can be visualized and from the fact that cytochalasin B, a compound impeding the formation of actin filaments, prevents phagocytosis.

The mechanism of force generation during the phagocytosis is still an enigma. It could be driven

- (i) by the polymerization of actin and simultaneous bundling of the filaments into so called stress fibres at the leading edge of the lobe which pushes the membrane forward [25], or
- (ii) by the spontaneous spreading of the plasma membrane of the phagocyte over the target cell which triggers the ATP-driven formation of the actin-gel layer in the advancing lobe, cf. fig. 23, thus stabilizing the engulfment.

Whatever the mechanism, due to the concerted adhesion of the plasma membrane and formation of an actin cortex, phagocytosis is a typical membrane process.

7.2. *On the export of cellular organelles by budding and secretion*

Budding is important not only for the trafficking of intracellular material but also for the export of large organelles. An interesting example is again found during the maturing of erythrocytes. The nucleus is excluded by budding, followed by fission of the nucleus containing vacuole (cf. fig. 24a). The removal of residual organelles and debris of the newly born cells, also called reticulocyte, occurs by secretion. First a large vacuole is formed within the cell. It fuses with the plasma membrane and the content is expelled. This process is impressively demonstrated in fig. 24b.

8. **On the interplay between fast randomization and local phase separation in biomembranes**

Biological membranes are in general in a fluid state and cells try to adjust their lipid composition to maintain this state. Judging from calorimetric studies of natural lipids (cf. chapter 5) lateral phase separation is, however, possible at physiological temperatures in the presence of substantial amounts of long chain lipids (e.g., sphingomyelins). As suggested above, local phase separation may help in the sorting of membrane components during fission of vesicles from endosomes, CURL-vesicles and secondary lysosomes. More importantly, local lateral phase separation enables the formation of functional units within membranes.

Phase separation in membranes is often observed in cases where large numbers of proteins are required for a certain function. One example is the formation of aggregates of proton pumps within epithelial cell membranes of the bladder or kidney (as shown in the example of fig. 25). Another example is the quasicrystalline arrays of bacteriorhodopsin in halobacteria. A more subtle example is the thylakoid membrane of chloroplasts. The distribution of the two photosystems is non-random:

Fig. 25. Example of local clustering of functional units in animal cell membranes. Electron micrograph showing a quasi-crystalline array of proton pumps (V-class H^+ -ATPases) in plasma membrane (apical side) of toad bladder epithelial cell. Each particle is a single ATP-ase (600000 Daltons). (Source: D.Prown, S.Gluck and J.Hardwig, *J. Cell. Biol.* **105**, 1637 (1987).)

photosystem II is more abundant in the grana than in the stroma and the reverse holds for photosystem I (cf. [2]).

Since cells try to maintain their membranes in the fluid state and therefore the lipid composition and the lipid/protein ratio are adapted to environmental conditions. The best known example is plant cells, which are well known to adjust the chain length and degree of unsaturation of the phospholipids to the environmental growth conditions (cf. [24] for references). Thus, in order to cultivate cold-resistant plants one has to increase the relative amount of highly unsaturated lipid. Another well documented example is the growth of mycoplasmas, small bacteria-like organisms living in a parasite association with animal and plant cells. Studies of mutants (so called auxotrophs) which cannot synthesize lipids and in which specific chain lengths can be accumulated by addition of the corresponding fatty acids to the nutrition medium show that these mutants can only grow and divide at temperatures above the chain melting transition of their components.

One reason for this adaption of the lipid structure to the environmental condition is that the enzymatic activity of integral membrane proteins is strongly impeded if the lipid is cooled below its main phase transition. This is the reason for the well known breaks in the apparent activation energy of the enzymatic processes which appear at the liquidus lines of the lipid mixtures in which the enzyme resides (cf. reference [26] and chapter 5). In natural membranes the breaks can be shifted to low (or high) temperatures by adding short or unsaturated (or long and saturated) lipids to cells.

8.1. Diseases are often associated with changes in lipid composition

Concerning the question whether the complex lipid composition of biomembranes is biologically essential, it is important to realize that diseases are often accompanied

by characteristic changes in the lipid composition. One example is diabetes. The cholesterol-to-phospholipid molar ratio of erythrocyte membranes is increased from 0.9 for normal donors to 1.4 for patients with diabetes. This is most certainly a consequence of a parallel change of this ratio in the cytoplasm which is 2.9 for normal donors and 3.1 for patients. Since cholesterol is rapidly exchanged between the aqueous phase and membranes the changed composition is expected to hold also for other cells in continuous contact with the blood.

Another more remarkable example is schizophrenia. In erythrocytes of schizophrenic patients the PS content is increased drastically from $12 \pm 2\%$ to $18 \pm 3\%$ at the expense of PC and PE [27]. It is again likely that similar changes hold also for the synaptic membranes. The changes in PS composition lead to a remarkable increase of the surface charge of the cytoplasmic leaflet which could well influence the Ca^{++} -induced fusion of the synaptic vesicles with the presynaptic membrane.

A most dramatic example of a disease caused by perturbations in lipid composition is the Tay–Sachs syndrome (cf. [2], chapter 23). This genetic disease is caused by the absence of an enzyme, β -N-acetylhexosaminidase, which removes N-acetylgalactosamine residues from gangliosides (see fig. 1 for structure). The enzyme is one member of a family which cleave the various sugar groups of the gangliosides in a stepwise manner, and by the failure of one member of the family of enzymes the degradation of gangliosides is impeded. Since this process takes place within the lysosomes, the gangliosides accumulate in these organelles forming multilamellar lipid particles. The gangliosides are most abundant in nerve cells (about 6% of total lipid) and therefore the defect results in severe mental deficiency and is lethal about three years after birth.

The degradation of gangliosides involves another interesting aspect. Several of the enzymes cannot attack the lipids while they are incorporated in the membrane and require auxiliary proteins in order to exert their function. This behavior is in striking contrast to the decomposition of phospholipids by phospholipases. These are small glycoproteins which pull the gangliosides out of the membrane to present them to the degrading enzymes. Genetic defects resulting in the failure of these cofactors lead to dramatic and lethal brain defects [28].

8.2. *Two-dimensionality of membranes is essential for diffusion controlled processes and enables manipulation of lateral protein mobility over orders of magnitude*

The two-dimensionality of the membranes offers many advantages for several reasons.

Firstly, diffusion controlled processes may become much more effective in 2-dimensional than in 3-dimensional space. Thus the average time τ_R for a reactant to reach a target site of diameter a at a distance b from the reactant depends drastically on the dimensionality ([29]). It is of the order of

$$\tau_R^{(3)} \approx \frac{b^3}{3Da} \quad (5a)$$

in three, and

$$\tau_R^{(2)} \approx \frac{b^2}{2D} \ln \frac{b}{a} \quad (5b)$$

in two dimensions where D denotes the diffusion coefficient. Three body collisions required for enzymatic reactions occur extremely seldomly in solutions but may become quite effective in two dimensions.

A second advantage is the logarithmic dependence of the lateral diffusion coefficient on the radius R_p of an integral membrane protein. For that reason, large integral proteins exhibit diffusion coefficients in model membranes of the same order of magnitude as lipids, as verified in many model membrane studies (cf. [33]).

One example for which fast mobility of proteins is essential is the electron transfer chain of the inner mitochondrial membrane. Extensive electron microscopic studies and diffusion measurements have provided evidence that the overall kinetics of electron transfer is largely diffusion controlled [30]. The four integral protein complexes involved (NADH-ubiquinone oxidoreductase, succinate ubiquinone oxidoreductase; ubiquinol-cytochrome *c* oxidoreductase, cytochrome oxidase) are randomly distributed and about half of the area of the bilayer of the inner membrane is composed of lipid. The rate of electron transfer between any two enzymes of the electron transfer chain can be accounted for by assuming that it is determined by the long range diffusion of the two reactants. The protein diffusion coefficients vary between $D_p = 2 \times 10^{-10}$ and 8×10^{-10} cm²/sec whereas the small electron carrier ubiquinone diffuses much more rapidly ($D_L = 2 \times 10^{-8}$ cm²/sec).

A third unique advantage of the two-dimensionality of membranes is that the lateral mobility of proteins can be controlled over many orders of magnitude. A prominent example is the band III diffusion in erythrocytes. A mobile and an immobile fraction are found and their ratio depends drastically on the state of the membranes. Thus, the mobile fraction is only 10% at 21°C (with $D_p = 4 \times 10^{-11}$ cm²/sec) but 90% at 37°C (with $D_p = 2 \times 10^{-9}$ cm²/sec [31]). In fact, a whole distribution of mobilities of integral proteins has been reported for LDL-receptors [32]. These findings can be explained by two coupling mechanisms of the cytoplasmatic domains of the membrane proteins to the cytoskeleton. Firstly, the proteins may be practically immobilized by covalent linkage (e.g., of the LDL-receptor to the clathrin coat mediated by α -adaptin). Secondly, the mobility of membrane-bound macromolecules may be drastically reduced even by frictional coupling of the diffusant to the cytoskeleton elements. If the former is separated from the latter by only a very thin water film (of thickness $h_w \approx 1$ nm) the logarithmic law breaks down and D_p can become quadratically dependent on the protein radius according to [33]:

$$D_p \approx \frac{k_B T h_w}{\pi \eta_w R_p^2} \quad (6)$$

where η_w is the water viscosity. This law has been verified for model membranes exhibiting frictional coupling to solid supports. It provides a powerful method to evaluate the rheological properties of lipid membranes or to measure 2D-hydrodynamic radii of integral proteins or macrolipids.

Another reason for the large variability of long-range diffusion coefficients of proteins in membranes is their mosaic-like organisation into fluid and gel-like domains. This micro phase separation becomes of course most important at low temperatures. It is most probably the main reason for the above mentioned large fraction of immobilized band III protein in erythrocytes membranes at 21 °C since lateral phase separation sets in at $T < 20^{\circ}\text{C}$.

8.3. Hormone signal transduction: a possible example of the interplay between rapid lateral randomization and local aggregation

Microphase separations and their possible role for the formation of specialized regions in biomembranes is one of the great issues of membrane research. One possible example where it may be exploited for the control of membrane processes is the down-regulation of hormone activity. This complex membrane process will therefore be discussed in the following.

Signal transduction based on c-AMP-amplification. Water soluble hormones regulate many intracellular reactions in eucaryotic cells. Examples are:

- (i) epinephrine hormones belonging to the class of β -adrenergic receptors which evoke a rapid increase of the glucose level in the blood;
- (ii) erythropoietin, which triggers the differentiation of stem cells in the bone marrow to the nucleated precursor cells of erythrocytes called the erythroblasts (cf. [3], chapter 16).

These hormones are recognized by specific receptors (see fig. 26). Each cell carries several types of such receptors. However, each type is present in very small quantities, typically about 10000. One stem cell for instance contains only 1000 erythropoietin receptors. Therefore amplification mechanisms are required for hormone action. The amplification mechanism consists in the production of signal substances such as c-AMP in the case of β -adrenergic receptors. This ubiquitous second messenger modifies the activity of many cellular enzymes by triggering their phosphorylation, a process mediated by kinases.

The hormone signal transduction is a genuine membrane process. The machinery consists of three proteins (see fig. 26): (1) the hormone receptor, (2) the adenylate cyclase which acts as the catalytic unit for the c-AMP production and (3) the G-protein which transmits the signal from the receptor to the catalytic unit. All β -receptors exhibit the same basic structure of seven membrane-spanning-helices with the specific binding site extending into the extracellular space. The cytoplasmatic hydrophilic loop between the helices 5 and 6 is rather large and acts as binding site for the G-proteins (the signal transducer).

The G-protein is a complex of three peptide chains: G_{α} (42kD), G_{β} (35kD) and G_{γ} (10kD). The α and γ units are known to couple the G-protein to the lipid bilayer via lipid anchors. The α contains the binding site for GTP and GDP which are both bound non-covalently. The key property of the G-protein for signal transmission is that it exists in a resting and an activated state. In the resting state GDP is bound to the G_{α} -unit (called GDP- G_{α}) which is then closely associated with the β and γ oligomers. The G-protein is activated by replacement of GDP by GTP (a receptor

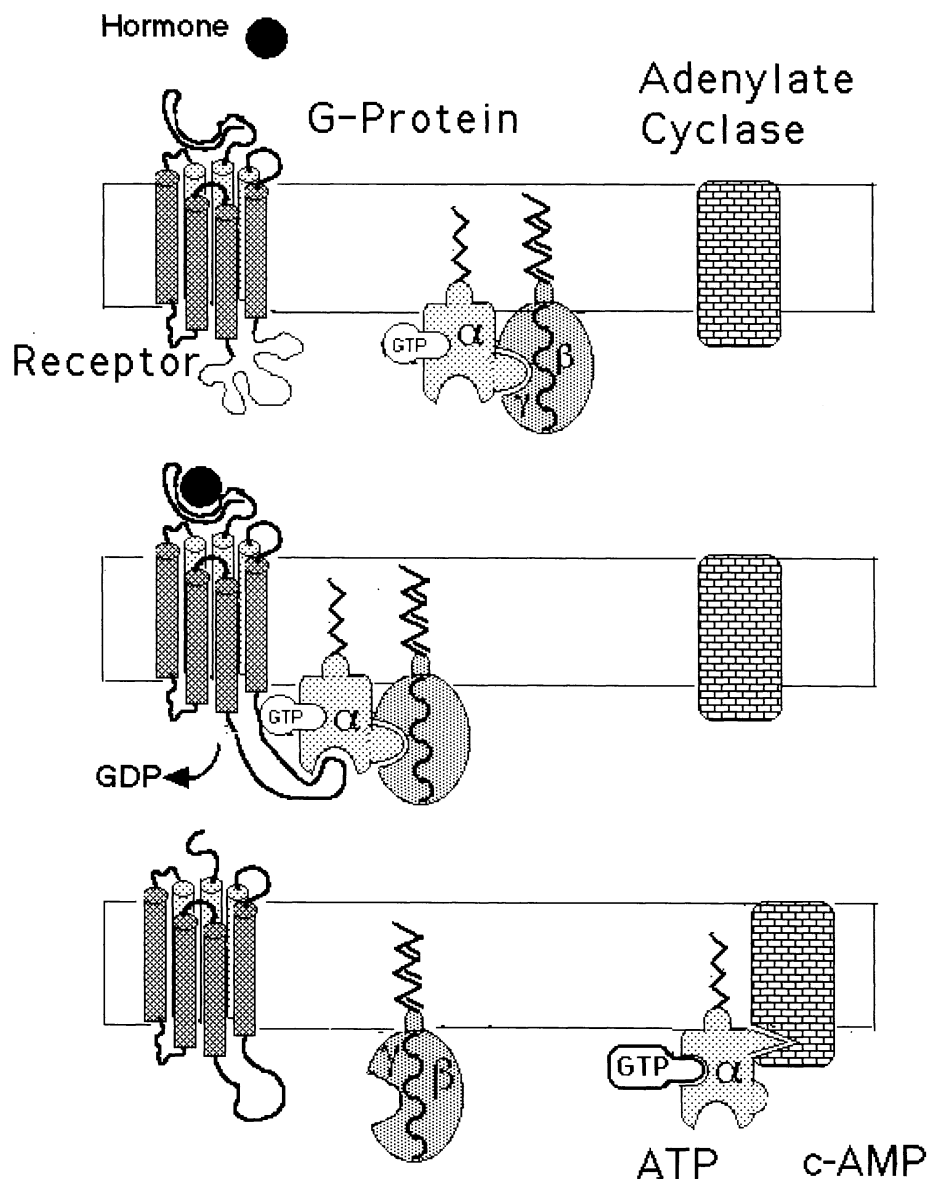


Fig. 26a. Schematic model of hormone signal transduction and amplification by machinery consisting of three proteins: (1) the β -adrenergic receptor, (2) the GTP-regulated G-protein and (3) the adenylate cyclase (AC). The receptor consists of an integral protein spanning the membrane with seven helices. The G-protein is a trimer composed of an G_{α} , G_{β} and G_{γ} unit of which two are coupled to the bilayer by lipid anchors. The binding site of the G-protein to the receptor is located at the cytosolic loop between helix number 5 and 6. Receptor and G-protein are similar in visual signal transducer of rod cells.

mediated process). The activated GTP- G_{α} -unit is released from the trimeric-complex

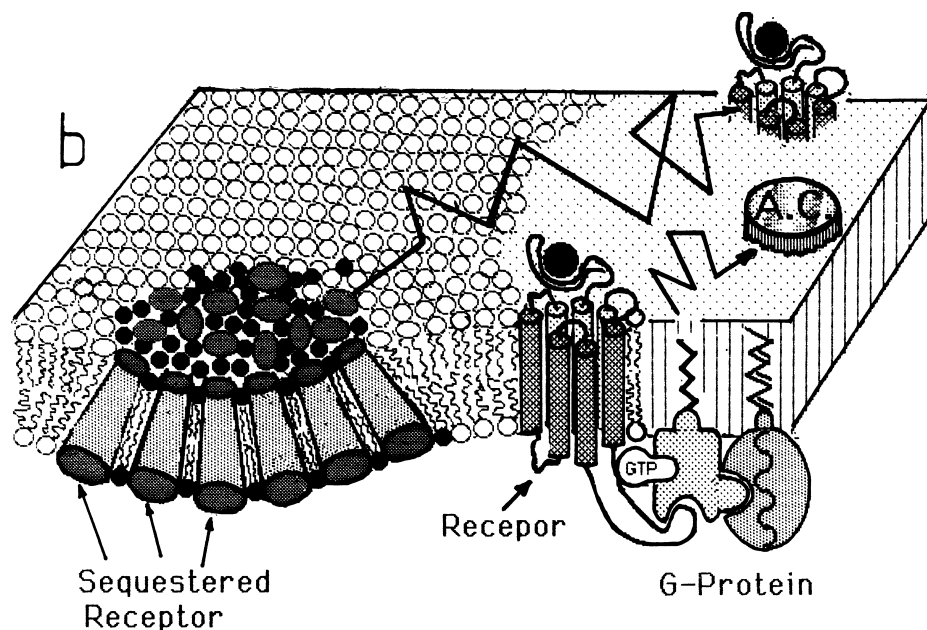


Fig. 26b. Local clustering of receptors in the plasma membrane as a possible mechanism of downregulation of hormone activity by sequestering and de-activation of receptors. Note that both the coupling of the G_{α} -unit to the activated hormone and the transfer of the GTP- G_{α} -unit to the adenylate cyclase are diffusion controlled processes.

and binds strongly to the adenylate cyclase, thereby triggering the production of c-AMP from ATP.

The hormone signal transduction is a diffusion controlled process and consists of the following sequence of events: (1) binding of hormone to receptor; (2) induced coupling of G-protein (in resting state: GDP- G_{α}) to receptor (a process triggered by a conformational change of the receptor); (3) induced exchange of GTP for GDP followed by release of GTP- G_{α} from ($\beta\gamma$)-unit; (4) diffusion of GTP- G_{α} to adenylate cyclase to which it couples and induces the production of c-AMP.

The G-protein mediated signal transduction is a universal process in biomembranes. Most remarkable is the close analogy between the β -hormone signal transmission and the sensory transduction in the visual and olfactory systems (see references [1], chapter 20, and [36] for details). The visual signal transduction machine is situated in the membranes of the discs which are located within the rod cells. Light induces a hyperpolarization of the cell. The role of the receptor is played by rhodopsin, that of adenylate cyclase by a phosphodiesterase and the second messenger is c-GMP (cyclic guanosine monophosphate) instead of c-AMP. The G-protein is called transducin which is very similar to other G-proteins. The rhodopsin exhibits the same type of structure as the β -receptor, consisting of seven membrane-spanning helices. The large hydrophilic loop separating helix 5 and 6 acts as the binding site for the G-protein. The signal transduction results in the decomposition of c-GMP

by the phosphodiesterase after its activation by the transducin enzymatic unit. The reduction of the c-GMP level in the cytosol of the rod outer segment induces the hyperpolarization of the rod cell membrane. The sequence of events is: (1) activation of rhodopsin by light-induced dissociation of retinal; (2) binding of resting state G-protein (with GDP- G_{α} -unit) to opsin (the retinal depleted rhodopsin) which triggers replacement of GTP for GDP; (3) dissociation of GTP- G_{α} -unit and coupling to phosphodiesterase. The latter process is again determined by the lateral diffusion of the GTP- G_{α} -unit to the enzymatic unit.

The strength of the hormone action can be regulated (1) biochemically (e.g., by phosphorylation), (2) by variation of the population of hormones and receptors and (3) by the accessibility of the receptors. Finally, the c-AMP level in cells is regulated by the counteraction of the adenylate cyclase and the c-AMP decomposing phosphodiesterase. The action of the latter is in turn modulated by Ca^{++} .

Hormone specificity and signal amplification. Nature's strategy to use many hormone-specific receptors but an universal signal transducing system requires a high specificity of hormone-to-receptor binding. The dissociation constant of the hormone-receptor complex is indeed very small: for instance $K_D = 10^{-8}$ M for insulin and $K_D \approx 10^{-11}$ M for erythropoietin. A second crucial point is that the signal transduction is a diffusion controlled process. It is made possible by the high efficiency of such processes in two dimensions.

Physiological response requires the production of 10^4 to 10^6 c-AMP molecules per receptor hormone binding event. A high amplification factor of the signal transducing system is thus required. A first stage of amplification is the conversion of many G-proteins by each hormone-receptor complex (by replacing GTP for GDP in the G_{α} -unit). Thus, one opsin molecule (retinal depleted rhodopsin) can activate about 500 G-proteins (= transducin) molecules. Similar numbers hold for the β -receptor. To our present knowledge, one GTP- G_{α} subunit activates only one adenylate cyclase unit which (in the activated state) can produce about 10^4 c-AMP molecules. This results in an amplification factor of the order of 10^6 .

Down regulation. Continuous presence of the hormones is required to produce c-AMP. However, exposure of cells to a high level of hormones for hours renders the receptor inactivate. This so-called downregulation is essential to prevent overactivation. How is this overactivation prevented? Several mechanisms have been found:

- (i) First the ability of hormone receptors to transduce hormone signals to G-protein is reduced by phosphorylation. This regulation mechanism occurs in a negative feed-back process by activation of kinases (which phosphorylate the receptor) by c-AMP.
- (ii) Certain hormones cause a decrease of the c-AMP level. This inhibition is mediated by a different G-protein, the α -unit of which inhibits the adenylate cyclase activity. This enables the fine tuning of the hormone action similar to the co-action of excitatory and inhibitory synapses in neural systems.

(iii) The long time regulation occurs by variation of the number of receptors in the plasma membrane which are accessible to the hormone.

The latter mechanism is a beautiful example of a regulation via the number of active elements of a signal transducing system. It is furthermore a possible example of an interplay between fast randomization and lateral phase separation in membranes leading to membrane instability. The regulation of membrane processes by numbers will be considered now more closely:

Let K_D be the hormone-receptor dissociation constant. It can be easily verified by application of the law of mass action that the fraction of occupied receptors is (cf. reference [1], chapter 19)

$$\frac{[\text{HR}]}{[\text{HR}] + [\text{R}_0]} \approx \frac{[\text{HR}]}{\text{R}_0} = \frac{1}{1 + K_D/[\text{H}]} \quad (7)$$

where R_0 , $[\text{H}]$ and $[\text{HR}]$ are the concentrations (1) of the accessible receptors in the membrane, (2) of the hormone and (3) of the receptor hormone complexes, respectively. Provided the minimum number of occupied receptors required to evoke an action is $[\text{HR}]^*$, the minimum hormone concentration must be

$$[\text{H}]^* = \frac{[\text{HR}]^* K_D}{[\text{R}_0] - [\text{HR}]^*} . \quad (8)$$

This is a hyperbolic law which implies that the number of hormones required can depend very sensitively on the number of available receptors. Near the threshold $[\text{R}_0] = [\text{HR}]^*$ a small reduction of available receptors can require a dramatic increase in hormone concentration in order to induce a signal transduction. The hormone concentration in the blood plasma is adjusted in such a way that the fraction of occupied receptors $[\text{HR}]/[\text{R}_0]$ is about 50% (cf. also reference [1], chapter 19). A small increase of the receptor concentration can shift the $[\text{H}]^*$ -versus- $[\text{R}_0]$ curve in the diverging regime of the hyperbolic curve.

Various studies suggest [34] that hormone action is down-regulated by the reduction of the number of receptors in the plasma membrane. This occurs most probably by lateral segregation followed by internalization. This process is modeled in fig. 26. It is not clear whether this receptor sequestering procedure is due to endocytosis via coated pits or pinocytosis. On the other hand, evidence was provided in the above work that the receptors remain closely associated with the plasma membrane. Thus sequestering of receptors by lateral phase separation without internalization is another likely possibility.

Abbreviations

Chemicals

PC: Phosphatidylcholine,
PE: Phosphatidylethanolamine,

PS: Phosphatidylserine,
 SPHM: Sphingomyelin,
 DMPC: Dimyristoylphosphatidylcholine (synonymous for other lipids of the PC class),
 CURL: (= compartment of uncoupling of receptor and ligand): Vesicle of bi-directional pathway of trafficking between Golgi and plasma membrane,
 ER: Endoplasmatic Reticulum.

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