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The Fluid Double Polar-Nonpolar-Polar Leaflet Model for Biological Membranes

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ABSTRACT

A model for biological membranes is proposed according to which the plasma membrane consists of two functionally different polar-nonpolar-polar leaflets separated by a polar space. The binding of water-soluble proteins, integral lipoproteins and spanning proteins to a biological membrane as well as possible conformations of interphase peptides partitioned between polar and nonpolar layers are discussed. A model for the diffusion of water soluble proteins across nonpolar layers of a membrane is described. Three complete biological membranes containing two leaflets and an inter-leaflet space are defined. These are: 1: The inner nuclear membrane + the perinuclear space and the endoplasmatic cisternae + the outer nuclear membrane and the endoplasmatic reticulum, 2: the inner mitochondrial membrane + the mitochondrial intermembraneous space + the outer mitochondrial membrane and 3: the cytoplasmic leaflet of the plasma membrane + an intramembraneous space in the plasma membrane + the outer leaflet of the plasma membrane.

INTRODUCTION

The fluid mosaic model proposed by S.J. Singer and G.L. Nicholson in 1972 (46) represented considerable progress in our understanding of several plasma membrane phenomena but it does not satisfactorily explain all of them, in particular several findings that have been made in this laboratory during the past 10 years relating to the association of cytoplasmic enzymes to the plasma membrane (41), albumin-inhibited leakage of such enzymes into the extracellular medium (54) and the apparent existence of an intramembraneous pool of solutes (4). All cells hitherto investigated in this laboratory display extracellular adenylate metabolism including ATP synthesis (1, 5, 42), adenylate kinase (5), NDP kinase (2), ATPase (3, 43), and protein kinase (6, 44). Phenomena such as ghost resealing (*cf.*: 26), secretion of proteins apparently without exocytosis (23, 32), and the transport of polynucleotides across intact plasma membranes (*cf.*: 36) are difficult to explain with the traditional lipid bilayer concept of a plasma membrane. This concept for biological membranes in general has previously been questioned on morphological (47), chemical (28) and biofunctional (28) grounds. Perhaps the most convincing objection is that lipids can be extracted from some biological membranes without altering the usual trilaminar appearance in electronmicroscopic pictures (18). Also the nature of the material in the middle sheet which does not stain with osmium remains undetermined because according to the traditional view strong hydrophobic bonds would be expected to be present thus preventing, for example, tangential splitting of this region on freeze-etching (*cf.*: 51).

THE MODEL

As an approach to these and other questions associated with the traditional lipid bilayer concept, the fluid double polar-nonpolar-polar leaflet model for biological membranes illustrated in Fig. 1 to 7 is proposed. According to this model, biological membranes consist of two functionally different polar-nonpolar-polar (PNP) leaflets each containing water soluble and integral (46) proteins, lipids, and extended polypeptides partitioned between polar and nonpolar phases. One leaflet is in addition enriched in carbohydrate residues shared by the polar and nonpolar layers. The structure of the plasma membrane, for example,

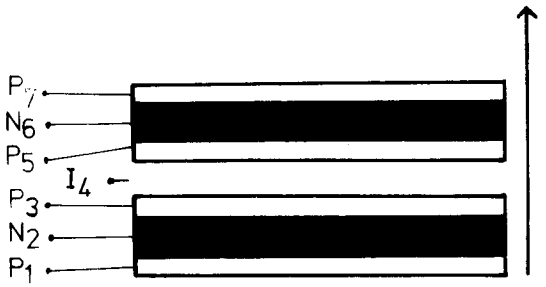

Fig. 1. The principal structure of a biological membrane. Light areas indicate polar and dark areas indicate nonpolar residues (P = polar layer, N = nonpolar layer, I = intermembraneous space). The indices and the arrow indicate the polarity of the two leaflets. The proportions between the different layers and the intermembraneous space are not necessarily the same as in this illustration, but may depend on the composition and the amount of the different chemical constituents.

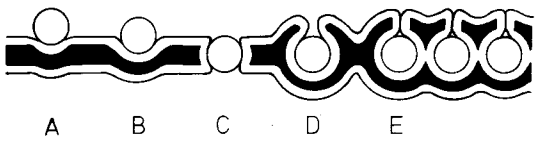

Fig. 2. Association of watersoluble proteins to one leaflet of a biological membrane. The capitals A–E indicate increased complexity of the interactions between associated proteins and the membrane.

from the cytoplasmic surface and outward is given by the following general scheme (see Fig. 1).

The layers of the N2 leaflet

P1. Polar bound (= associated and covalently bound to the nonpolar layer) residues.
N2. Fluid (9) nonpolar residues.
P3. Polar bound residues.

The I4 space

Intermembraneous polar space containing solutes and enzymes but susceptible to conventional electron microscopic preparatory techniques.

The layers of the N6 leaflet

P5. Polar bound residues.
N6. Fluid nonpolar residues.
P7. Polar bound residues.

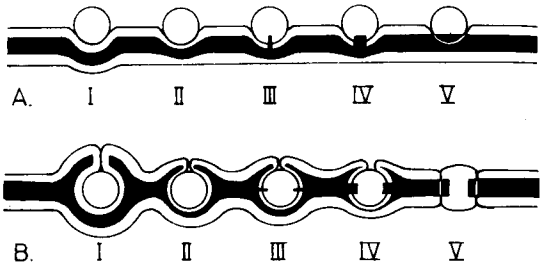

Fig. 3. Development of watersoluble membrane-associated proteins into integral (A–B) and spanning (B) proteins. The Roman numbers I–IV indicate increased content of nonpolar amino acid residues involved in binding the integral protein to a membrane.

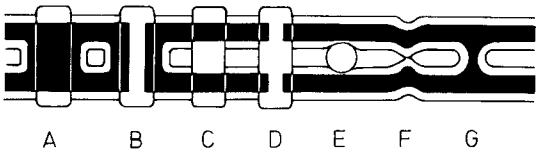

Fig. 4. Possible modes of binding of an integral protein spanning (46) across two leaflets of a biological membrane e.g. the plasma membrane (A–D). Stabilization of close spatial arrangement of two leaflets of a biological membrane by indirect (E) and direct (F) polar bonds and by fusion of the N2 and N6 leaflets (G).

The binding between a membrane and globular proteins that do not become denatured when extracted (e.g. amino acid-binding proteins from bacteria, 10) is illustrated in Fig. 2. The substitution of polar bonds involved in this type of binding for nonpolar bonds resulting in proteins integrated (46) in the nonpolar layer (e.g. a Na^+- and-K^+-stimulated ATPase, 10) is illustrated in Fig. 3 A and B. Two types of spanning (46) proteins can be visualized in this model, mainly polar-nonpolar-polar (PNP, see Fig. 4A and B) and mainly polar-nonpolar-polar-nonpolar-polar (PNPNP, see Fig. 4 C and D) tentatively illustrated by glycophorin A (35) and the band 3 protein (25, 35) from erythrocytes respectively. The spanning proteins presumably hold the two leaflets of the plasma membrane together. This function could also be accomplished by indirect or direct polar bonds between the P3 and P5 layers as illustrated in Fig. 4 E and F or by fusion (*cf.*: 29) of the N2 and N6 layers (Fig. 4 G).

It is to be expected that a PNP leaflet heavily

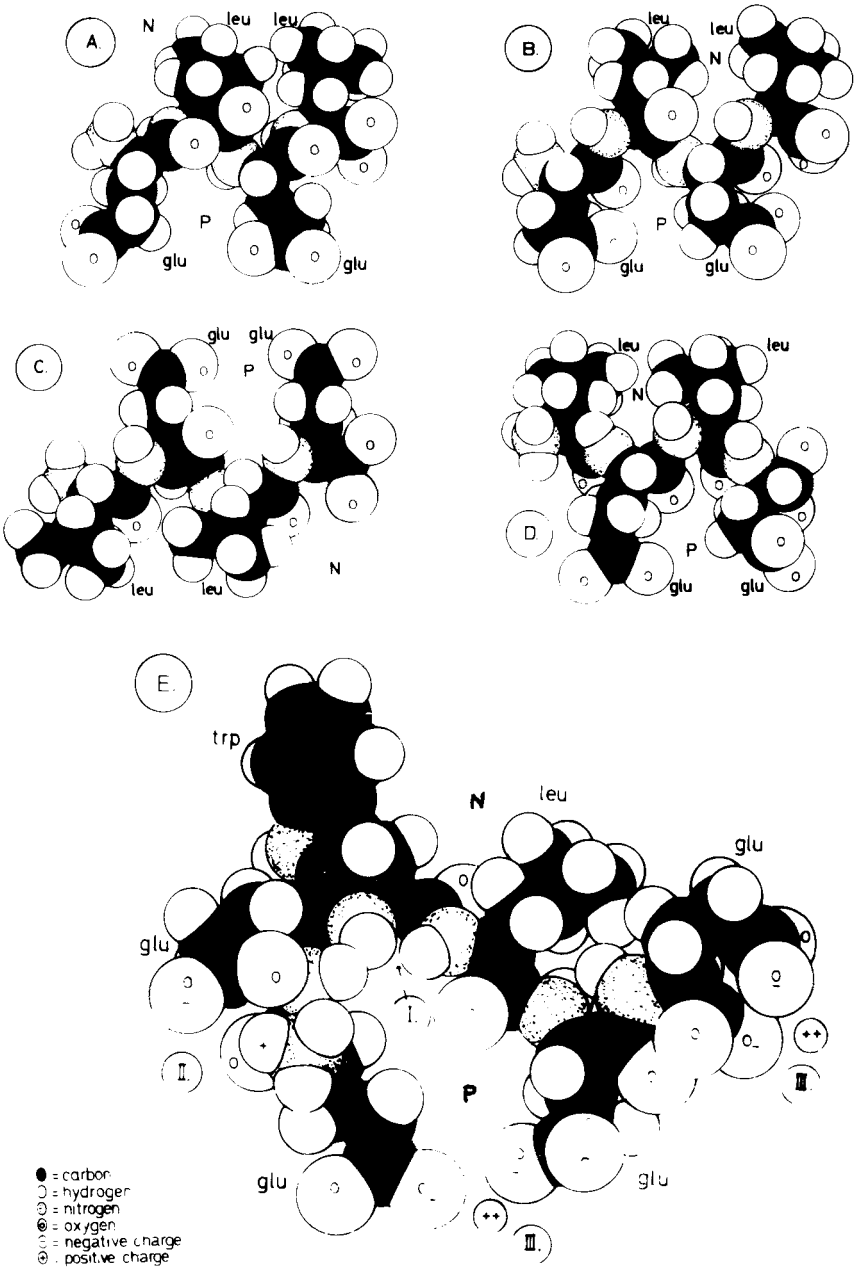

Fig. 5. Conformations of oligo-peptides partitioned between polar (P) and nonpolar (N) layers of a membrane without reference to possible stabilizing interactions with adjacent molecules. Oligopeptides with alternating polar and nonpolar amino acids represented by glu-leu-glu-leu (A—B) and leu-glu-leu-glu (C—D). Part of a ring structure (E I) and possibilities for direct (E II) or indirect (E III) electrostatic stabilization illustrated by a conformation of the oligopeptide glu-glu-trp-leu-glu-glu.

loaded with electronlucent and possibly displaceable enzymes bound as illustrated in Fig. 2 E would yield an electron microscopic picture reminiscent of a lipid bilayer. The reason for this is that polar and nonpolar residues of a membrane are closer and therefore firstly more susceptible to cross-linking, secondly more electron dense and thirdly, since they are linked together by both polar and nonpolar bonds, also less extractable. However, on careful examination this type of trilaminar membrane (see Fig. 2E) would be expected to reveal globular structures resembling those found in mitochondrial and endoplasmatic reticulum membranes (47). The profound effect on the interleaflet distance in biological membranes of conventional electron microscopic preparatory techniques (48) which are known to produce leakage of cytoplasmic proteins, shrinking and swelling of specimens, is documented in the case of mitochondria (49) chloroplasts (50) and plasma membranes (17). In all these cases, certain preparation techniques seem to preserve a broader interleaflet distance. This favours the view that inter-leaflet spaces are polar and excludes the possibility of strong nonpolar bonds in this region.

Tangentially extended polypeptides with polar residues directed outward and nonpolar residues directed inward in the proteolipid leaflets are imagined to constitute some kind of a lipid skeleton and in addition to mediate the attachment of watersoluble enzymes and carriers. These interphase polypeptides could be expected to require a secondary structure different from the α-helix, the β-pleated sheat and the random coil, perhaps making them even more susceptible to cross-linking agents used in electron microscopy and to other frequently used denaturing procedures. Some possible configurations of such interphase polypeptides are illustrated in Fig. 5.

As shown in Fig. 5 A—D, polypeptides with alternating polar and nonpolar residues are readily partitioned between the water and lipid phases of a biological membrane. Exclusively nonpolar peptides may form a ring structure with the nitrogen atoms of the peptide bond directed towards the center. Part of such a ring located in the interphase between polar and nonpolar layers is shown in Fig. 5 E. Numerous possibilities for stabilization of an interphase conformation by electrostatic interactions exist, some of which are illustrated in Fig. 5 E. These interactions could be direct, between positively and negatively charged amino acid residues, or indirect, mediated by cations or anions that link similarly charged groups together.

This model imposes functional similarities between the plasma membrane and the mitochondrial membranes because, in both cases, a pool of enzymes involved in adenylate metabolism (*e.g.* adenylate kinase, 5, 16, 33, and NDP kinase, 2, 33) is located in the I4 space. This pool is accessible from the outside through a N6 leaflet permeable to nucleotides (33) and containing carbohydrates (8, 37, 46). On the other hand the inner N2 leaflet is assumed to be impermeable to nucleotides necessitating the presence of various carriers in both inner mitochondrial (33) and plasma membranes. Phenomena associated with intact cells such as crypticity (15, 19) of enzymes (15, 19) and receptors (14) could be explained by this similarity.

Weak interactions between the P3 and P5 layers are imagined to catalyze the fluid state-dependent fusion of the N2 and N6 layers (see Fig. 4 G) resulting in nonpolar intermembraneous bridges. The fusion points are possible sites for diffusion of cytoplasmic enzymes across the plasma membrane as illustrated in Fig. 6 A. They could thus be responsible for the fact that albumin inhibits leakage of several cytoplasmic enzymes from some tumor cells without affecting or displacing other enzymes expressing their activity at the cell surface (54) indicating differential accessability of albumin to the plasma I4 and intracellular spaces of macromolecules. On the other hand, preferential and specific entry of globular proteins through one PNP leaflet must be considered (see Fig. 6 B) because some soluble enzymes are enriched in the mitochondrial I4 space (33) which because of continuous great changes of shape (21) can not have any fusion points between its two leaflets. If all transport of macromolecules across the plasma membrane not mediated by exo- and endocytosis (38) occurred via the I4 space any function of enzymes em-

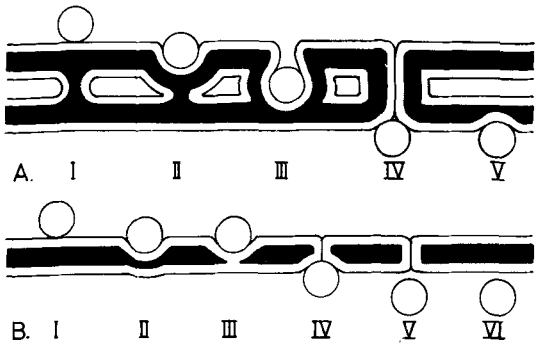

Fig. 6. Diffusion of watersoluble proteins across nonpolar layers of a membrane. The diffusion of a protein across a double leaflet membrane is shown in A. The Roman numbers I–III represent successive events while IV and V represent possible final events. Successive events in the diffusion of a globular protein across a single leaflet membrane are shown in B.

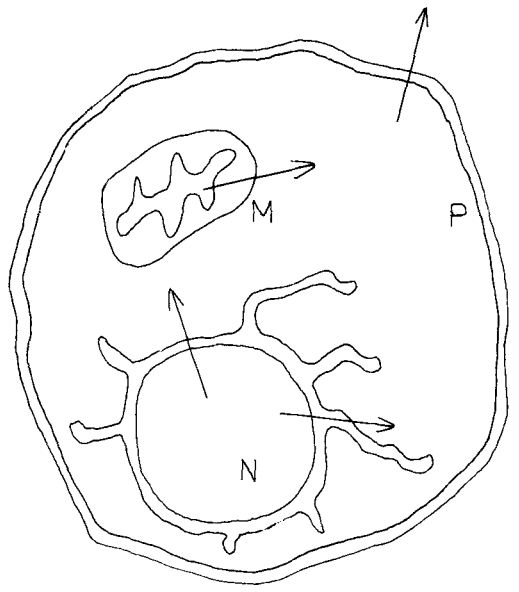

Fig. 7. The principal outline and polarity of three complete biological membranes, the inner nuclear membrane and the outer nuclear membrane combined with the endoplasmatic membranes, the mitochondrial membranes and the plasma membrane(s). The corresponding interleaflet spaces are the perinuclear space with the cisternae of the endoplasmatic reticulum, the intermembraneous space of the mitochondria and the intramembraneous space of the plasma membrane.

N=nucleus, M=mitochondrion, P=plasma membrane. The arrows indicate the polarity from the N2 to the N6 leaflet.

bedded in or between the two leaflets would be disturbed. This simple mechanism for transport of macromolecules across biological membranes implies that only adhesion sites could be required since low entropy adhesion would favour displacement of the underlying nonpolar fluid lipid residues.

On the basis of ultrastructural evidence and biochemical characterization the polarity of at least three complete biological membranes can be determined (see Fig. 7). By definition the association of energy transducers such as coupling factors (33) and actin (30, 31) and covalently bound DNA, characterizes a N2 leaflet as exemplified by the inner nuclear membrane (12, 13, *cf.*: 34) the inner mitochondrial membrane (33, *cf.*: 53) and the cytoplasmic leaflet of the plasma membrane (24 *cf.*: 22). Also the presence of adenylylcyclase (40) is a characteristic feature of some N2 leaflets. A comparatively high content of carbohydrates characterizes a N6 leaflet as for example the outer nuclear membrane (7, 37), the endoplasmatic reticulum (45), the outer mitochondrial membrane (8, 37) and the exterior leaflet of the plasma membrane (46). This characterization is supported by ultrastructural evidence indicating a globular appearance of the mitochondrial and endoplasmatic membranes (47) which resemble each other and are thinner than the plasma membrane (47). The facts that the nuclear membranes are thin and rarely display a trilaminar structure (52) indicate that these membranes will be possible to characterize as stacked with globular enzymes in analogy with the structure of mitochondrial and endoplasmatic membranes (47). The associated I4 spaces in these cases are the perinuclear space combined (52) with the cisternae of the endoplastmatic reticulum, the intermembraneous space of mitochondria and a corresponding intramembraneous space in the plasma membrane (4).

These three complete biological membranes containing N2 and N6 leaflets as well as an I4 space together with their polarity are illustrated in Fig. 7. The nature and functions of the intermembraneous space with its associated enzymes and solutes is interesting in view of the fact that this space containing adenylate kinase and NDP

kinase enlarges during oxidative phosphorylation in rat liver mitochondria (21). Similar fluctuations of the I4 spaces of other biological membranes could be expected to be associated with their characteristic functions. The demonstration that the endoplasmatic cisternae also contain adenylate kinase and NDP kinase *in vivo* will support generalizations facilitating the interpretation of phenomena associated with any of these three complete biological membranes by their mutual analogy.

DISCUSSION

This model for biological membranes offers the possibility of a new approach to several plasma membrane phenomena. The involvement of GTP in adenylyl cyclase activity (39) is one example. The enhanced transport of amino acids across the nuclear N2 leaflet brought about by exogenous ATP (27) may according to this hypothesis correspond to similar phenomena in the I4 space and N2 leaflet of the plasma membrane. Furthermore, various effects of treating intact cells with phospholipase C and other exogenous enzymes may be interpreted in terms of increased exposure of the I4 space and the N2 leaflet of the plasma membranes.

The finding that certain amino acids apparently are deprotonated *during* transport across the plasma membrane (11) may indicate that under certain conditions the I4 space is capable of maintaining a concentration of univalent cations, different from that on either side of the enclosing PNP-leaflets and may have important implications for the function of the mitochondria and the endoplasmatic reticulum. In the former case, swelling of the I4 space is correlated with oxidative phosphorylation and in the latter case inhibition of protein synthesis by puromycin is known to cause swelling of the endoplasmatic cisternae (20). The possibility that carriers generate a sodium gradient primarily across the N2 leaflet is interesting in view of the knowledge that inward transport of many metabolites is correlated with comigration of sodium (10). Due to the fact that sodium participates in an electrochemical gradient (10), such an arrangement would be possible to detect as a transient positive potential when inserting an electrode into a cell. If sodium-dependent transport of solutes occurs primarily across the N2 leaflet, this transient positive potential would be detected primarily in cells that are characterized by a high rate of sodium-dependent transport and by an N6 leaflet that is semipermeable to sodium ions.

The compartment idea for the subcellular location of macromolecules and the idea of vesicles as the unique mediators of transmembraneous transport of macromolecules are concerned by this interpretation of the structure of biological membranes. Several apparently unrelated observations dealing with, for example, the location of serum proteins in the nucleus (55) and the leakage of cytoplasmic proteins from intact cells (23, 32, 54) may be accounted for by assuming that the nuclear and plasma membranes and probably other intracellular membranes as well are permeable to certain macromolecules by diffusion as implied in this membrane model. This permeability, is an intersting weakness of tumor cells because of the possibility of introducing macromolecular agents that are harmful for their proliferative metabolism.

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