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Topographical Differentiation of the Cell Surface

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I. INTRODUCTION

The most attractive model for the molecular organization of the generalized cell surface membrane at the present time is the fluid mosaic model proposed by Singer and Nicolson (1972). This model depicts most proteins embedded individually to varying extents within a phospholipid bilayer. The bulk of the lipid matrix is sufficiently low in viscosity at physiological temperatures so that the proteins are free to diffuse or rotate laterally within the plane of the membrane. In this simplified form, the fluid mosaic model pictorially suggests a series of unanchored buoys (proteins) randomly afloat in a sea of lipid.

While some cell surface membrane proteins may function in such a fluid organizational state, others clearly require anchorage, or

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transient or stable associations with other proteins or lipids (*cf.* Cuatrecasas, 1974; Staehelin, 1974; Schmitt *et al.*, 1975; Nicolson, 1976a). Thus, topographical differentiation within the cell surface membrane must be of vital physiological significance. This is readily evident when considering, for example, the molecular differentiation of the plasma membrane of a neuron at a synaptic junction, or the morphological specialization of the apical surface of an intestinal epithelial absorptive cell into microvilli, but considerably less apparent in the case of the surface membranes of such cells as erythrocytes, lymphocytes or fibroblasts.

The purpose of this review is to assemble evidence relating to the topographical differentiation of cell surfaces, its functional significance, and how this differentiation may be established, maintained, or regulated within a dynamic membrane matrix. As considered here, the cell surface consists of the surface membrane together with all directly associated extracellular- and cytoplasm-oriented elaborations. Topography is meant to signify the two-dimensional morphological configuration of the cell surface, as well as the interrelationships between individual macromolecular components in both the plane and the depth of the cell surface.

It seems appropriate to begin this discussion by first considering some basic aspects of the molecular organization of the cell surface.

A. BASIC CELL SURFACE ORGANIZATION

Most recent interpretations of membrane structure are fundamentally similar to the fluid mosaic model in proposing that the majority of proteins are partly embedded within (integral proteins), and the rest superficially attached to (peripheral proteins), a phospholipid bilayer (Wallach and Zahler, 1966; Lenard and Singer, 1966; Singer, 1971; Bretscher, 1973; Hendler, 1974; Steck, 1974; Marchesi, 1974). This basic concept evolved primarily from consideration of thermodynamic principles applicable to the interaction of proteins

and lipids in an aqueous solution (reviewed by Singer, 1971). All integral proteins, glycolipids, and phospholipids constituting membranes are presumably amphipathic to some extent; that is, separate domains of the molecules are either rich in nonpolar amino acid or fatty acid residues, or in ionic or polar groups. As a consequence of various thermodynamic factors, nonpolar groups tend to establish noncovalent hydrophobic interactions in a nonpolar environment, while ionic or polar groups prefer hydrophilic interactions. Accordingly, the most stable membrane structure (that is, at the lowest free energy state) is one in which the hydrophobic regions of the phospholipids (fatty acid chains) and proteins are sequestered away from an aqueous environment (within the center of the membrane), while hydrophilic groups are maximally in contact with water (at either the extracellular or cytoplasmic surface of the membrane). The result is a lipid bilayer with the polar groups at both surfaces and the fatty acid chains inwardly oriented.

Integral membrane proteins are extremely heterogeneous within the same membrane, and from one membrane to another (Green *et al.*, 1968; Kiehn and Holland, 1968; Rosenberg and Guidotti, 1969). They generally exist in combination with oligosaccharides (glycoproteins) and/or lipids (lipoproteins), and in some cases may require interaction with membrane lipids to express normal functional activity (Green, 1968; Coleman, 1973; Machtiger and Fox, 1973; Farías *et al.*, 1975; Giraud *et al.*, 1976). Depending upon molecular size and configuration, and the distribution of polar and nonpolar residues, integral proteins may be embedded within the lipid bilayer to varying extents, or may span the entire thickness of the bilayer (transmembrane configuration). Singer (1974a) has postulated that peripheral proteins may be bound to membranes by weak interaction with exposed hydrophilic portions of integral proteins, resulting in the formation of functionally distinct molecular complexes.

Although receiving relatively little attention to date, membrane constituents such as bound water and divalent cations may play considerable roles in establishing, maintaining or altering membrane

organization (Sonenberg and Schneider, 1977).

1. Transmembrane asymmetry

Considerable evidence has accumulated indicating that cell surface membranes are constructed asymmetrically relative to the two halves of the phospholipid bilayer (reviewed by Bretscher, 1973; Singer, 1973, 1974a; Bretscher and Raff, 1975; Nicolson, 1976a; Rothman and Lenard, 1977). The existence of transmembrane asymmetry is not surprising considering that one side of the membrane faces the external environment, while the other side is in contact with the cytoplasm. It would be expected, for example, that cell surface receptors for membrane-impermeable hormones would be located at the external surface, while membrane-bound enzymes affecting intracellular metabolism ought to be at least partly exposed to the cytoplasm.

Glycoproteins and glycolipids, or at least the oligosaccharide and sialic acid residues of these molecules, appear to be represented exclusively at the external surfaces of mammalian plasma membranes, according to the results of labeling inside-out (inverted) and right-side-out membrane vesicles by galactose oxidase tritiation (Steck and Dawson, 1974), or lysed membranes with ferritin-conjugated ligands (Nicolson and Singer, 1971; Singer, 1973; Nicolson and Singer, 1974). A general impression of how glycoprotein molecules are integrated into the cell surface membrane may be afforded by the studies of Marchesi and his colleagues (Marchesi and Andrews, 1971; Marchesi *et al.*, 1972; Tillack *et al.*, 1972; Segrest *et al.*, 1972, 1973; Marchesi, 1974; Marchesi *et al.*, 1976), and others (*cf.* Winzler, 1972), on the structural organization of the major glycoprotein within the human erythrocyte membrane. It has been established that this molecule, designated glycophorin, is 60% carbohydrate and 40% protein, has a molecular weight of approxiamtely 31,000 daltons, and is linearly amphipathic (Marchesi *et al.*, 1972; Segrest *et al.*, 1972). Glycophorin consists of a single polypeptide chain of 131 amino acids organized into three distinct domains (Tomita and Marchesi, 1975). The

NH_2-terminal portion (removable from the membrane by digestion of ghosts or intact cells with proteolytic enzymes) contains predominantly hydrophilic amino acids (64 residues) and all (about 16) of the oligosaccharide chains. This portion of the molecule protrudes from the membrane into the extracellular environment (Eylar *et al.*, 1962), and contains MN and ABO blood-group antigens and receptors for *Phaseolus vulgaris* phytohemagglutinin, wheat germ and *Agaricus bisporus* agglutinins, and influenza virus (Pinto da Silva *et al.*, 1971; Marchesi *et al.*, 1972; Tillack *et al.*, 1972; Fukuda and Osawa, 1973; Triche *et al.*, 1975). The next segment of the molecule (which can be isolated only by treatments that disrupt the integrity of the lipid bilayer) is extremely hydrophobic and tightly associated with membrane lipids. This portion, consisting of 32 nonpolar residues, is thus deeply embedded within the interior of the bilayer and may, in combination with other membrane components, constitute the intramembranous particles visible within freeze-cleaved membranes (Marchesi *et al.*, 1972; Tillack *et al.*, 1972; Segrest *et al.*, 1974; Yu and Branton, 1976) (see Sections II, A, 1 and III, C, 1 for further discussion of the relationship between glycophorin and intramembranous particles). The COOH-terminal segment of glycophorin consists of 35 predominantly hydrophilic residues. Evidence obtained from labeling unsealed erythrocyte ghosts with the acylating agent [^{35}S]-formyl-methionylsulfone methyl phosphate suggests that this portion of glycophorin is exposed at the cytoplasmic surface of the erythrocyte membrane (Bretscher, 1971). Glycophorin appears, therefore, to span the entire membrane.

On the basis of results obtained from radioisotope labeling of intact erythrocyte ghosts and inside-out and right-side-out vesicles derived from ghosts, it is apparent that the other major glycoprotein of human erythrocyte membranes, designated component *a* or *3* (molecular weight about 100,000), is the only other integral protein spanning the entire membrane and is also asymmetrical with sugar groups exposed only at the external surface (Bretscher, 1973; Steck, 1974). This molecule bears receptors for the plant lectins concan-

avalin A (Con A) (Findlay, 1974; Pinto da Silva and Nicolson, 1974) and *Ricinus communis* agglutinin (Triche *et al.*, 1975) and may serve as an anion (Bretscher, 1973) or glucose (Lin and Spudich, 1974) channel through the membrane. Nucleated cell membrane-spanning proteins identified include a mouse L cell protein, virus envelope glycoproteins, human HL-A antigens, and transport enzymes (Hunt and Brown, 1975; Rothman and Lenard, 1977; Walsh and Crumpton, 1977).

The degree of penetration of other integral proteins into the erythrocyte membrane lipid bilayer is apparently highly variable, and may be specific and characteristic for each molecular species (Steck, 1974). All of these components probably display some degree of asymmetric disposition within the membrane. Among the molecules localized specifically at the outer surface of the human erythrocyte membrane are the enzymes acetylcholinesterase (Burger et *al.*, 1968) and nicotinamide adenine dinucleotidase (Alivisatos *et al.*, 1956), Rh and histocompatibility antigens (Nicolson *et al.*, 1971a, b), and the ouabain-binding site for Na^+, K^+ -activated adenosine triphosphatase (ATPase) (Hoffman, 1966). Molecules detected only on the inner surface include the enzymes ATPase (Marchesi and Palade, 1967), protein kinase (Rubin *et al.*, 1973), and glyceraldehyde-3-phosphate dehydrogenase (Kant and Steck, 1973), and binding sites for the cyclic nucleotide adenosine 3':5'-monophosphate (cyclic AMP) (Rubin *et al.*, 1973). Functional molecules of the plasma membranes of other mammalian cells are also asymmetrically disposed (Bosmann, 1972; Roth and White, 1972; de Pierre and Karnovsky, 1974; Bernacki, 1974; Trams, 1974; Webb and Roth, 1974).

Among the peripheral proteins of cell surface membranes (see review by Singer, 1974a), one of the best characterized is spectrin, which is attached to the cytoplasmic surface of erythrocyte membranes (Nicolson *et al.*, 1971c). Spectrin can be isolated from ghosts by treatment with low ionic strength, divalent cation-free solutions (Marchesi and Steers, 1968; Mazia and Ruby, 1968; Guidotti, 1972). The intact molecule is a noncovalently-linked complex of two polypeptide chains, with molecular weights of 240,000 and 215,000, cor-

responding to membrane components *1* and *2* as distinguished by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Spectrin appears to be associated with actinlike molecules of molecular weight 43,000 (component *5*) (Tilney and Detmers, 1975). These three polypeptides form a reticulum of microfilaments that adheres to the erythrocyte membrane without forming strong bonds with lipids. Guidotti (1972) has speculated that spectrin polypeptides may possess muscle actin- or myosinlike contractile properties. Components *1* and *2* do have physical characteristics similar to muscle myosin, and component *5* (actin) can be polymerized into microfilaments which behave like muscle actin in binding heavy meromyosin (Singer, 1974a). There is also evidence that reversible phosphorylation of spectrin components is involved in cell shape changes, suggesting that the spectrin complex may possess metabolically controllable contractile properties (Weed *et al.*, 1969; Williams, 1972; Singer, 1974a). Recently, Weidekamm and Brdiczka (1975) identified Ca^{++}, Mg^{++} -activated ATPase associated with spectrin that may serve such a function. The possible role of spectrin in controlling lateral mobility of erythrocyte membrane components will be discussed in Section IV, A, 5.

Glycoprotein and glycolipid components of mammalian cell surface membranes do not appear to rotate along an axis parallel to the plane of the membrane (transmembrane rotation, or "flip-flop") to a significant extent, and probably do not at all. This is evident from chemical and radioisotope labeling of carbohydrate moieties on membrane ghosts (reviewed by Bretscher, 1973; Steck, 1974), as well as from electron microscopic observations of membranes labeled with ferritin-conjugated plant lectins, ligands which bind specifically to select sugar groups (Nicolson and Singer, 1971, 1974). Integral membrane polypeptides similarly retain a consistent orientation relative to the outer and inner surfaces of the membrane (Singer and Nicolson, 1972; Bretscher, 1973).

Bretscher (1973) has recently reviewed evidence pointing to at least a partial compositional asymmetry, or sidedness, of phospholipids comprising the bilayer of the erythrocyte membrane. Phosphatidylcholine, sphingomyelin and glycolipids, for example, are more