J. Membrane Biol. 77, 169–186 (1984)

The Journal of
Membrane Biology

Topical Review

Distribution of Transport Proteins over Animal Cell Membranes

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Introduction

When Singer and Nicolson (1972) proposed the "fluid mosaic model" of membrane structure, their hypothesis was supported by two important experimental results. The first was Frye and Edidin's (1970) observation that cell membrane-bound antigens of two different tumor cell types intermix rapidly when the two cells are fused. Intermixing was correctly attributed to diffusion of the antigens in the plane of the membrane and occurred at a rate close to what was hypothesized to be the limit imposed by the viscosity of the lipid bilayer. The second finding was that integral membrane proteins in red blood cells appeared to be distributed randomly (Nicolson, Hyman & Singer, 1971). Taken together, the two findings suggested to Singer and Nicolson (1972) that "... membranes are a kind of two-dimensional solution of globular integral proteins dispersed in a fluid lipid matrix, as embodied in the fluid mosaic model of membrane structure" (Singer, 1974). Today it is well established that most of the lipid in cell membranes is indeed in a fluid state at physiologic temperatures (*see* e.g., Peters, 1981), that many proteins are mobile and also that the mobility of some proteins in the plane of the membrane has physiologic significance. Examples are the diffusion-mediated aggregation of some peptide hormone receptors into micro-clusters as a prelude to endocytosis, and the metabolically driven collection of cell surface receptors into a "cap" when lymphocytes are exposed to multivalent ligands such as antibodies or lectins. However, the lateral mobility of membrane proteins has received so much emphasis that to the casual student of cell biology, it may seem a general feature of animal cells. Yet it is becoming clear that in fully differentiated tissues, a large fraction of membrane protein is neither freely mobile nor randomly distributed.

Thanks to a number of new techniques, foremost among them "Fluorescence Photobleaching Recovery (FPR)" (Peters, Peters, Tews & Bahr, 1974; Axelrod et al. 1976; Edidin, Zagyanski & Lardner, 1976, Jacobson et al., 1976), it has become an almost routine matter to measure the mobility of lipids and of many proteins in the plane of the cell membrane. Surprisingly, proteins in most FPR experiments show both mobile and immobile fractions. The "immobile" fraction – typically 1/3 to 2/3 – has a diffusion coefficient of $<10^{-12}$ cm^2/sec, the limit of detection in current FPR experiments. But even the mobile fraction redistributes with diffusion coefficients of only 10^{-10} to 10^{-11} cm^2/sec. Lipid probes diffuse much more quickly ($D<10^{-8}$ cm^2/sec; Schlessinger et al., 1977). Since molecular size is expected to have only a weak influence on diffusion coefficient (Saffman & Delbruck, 1975), the diffusion coefficients of most membrane proteins should be of the same order of magnitude as that of lipids. To a greater or lesser degree, the mobility of even the mobile membrane proteins is, therefore, restricted. Evidently, Frye and Edidin's (1970) finding of unrestricted mobility was atypical. The mechanisms for immobilization are not yet fully understood (*see*, e.g., Tank, Wu & Webb, 1982). Lateral mobility of proteins has been discussed in several recent reviews (e.g., Cherry, 1979; Peters, 1981).

With regard to the distribution of integral membrane proteins, it has been known for some time that in fully differentiated tissues, membrane proteins may be highly localized. For example, the nicotinic acetylcholine receptors on skeletal muscle fibers are concentrated beneath the innervating nerve terminal (e.g. Miledi, 1960), as are receptors

Key words membranes · transport proteins · mobility · epithelia · nerve · muscle

for other neurotransmitters in other postsynaptic membranes. It was perhaps unclear at the time whether this discrete distribution of AChR was an unusual occurrence in cell biology. For example, Singer (1974) wrote "It seems likely now that these ordered structures are special in that they consist of a single protein, or very few species of protein molecules, which form a large two-dimensional regular lattice ..." However, others have suggested that segregation and immobility of membrane proteins may be fairly common: "A consideration of tissue architecture and cytochemistry quickly suggests that there must be mechanisms acting to restrict mobility of membrane molecules when cells are organized into tissues." (Edidin, 1972). Indeed, as fluorescent and radiolabeled ligands to membrane proteins became available and as morphologic and electrophysiologic methods improved, lateral segregation of membrane proteins was found in many organized tissues. This review aims to (a) list instances of segregation of membrane transport proteins, (b) discuss physiologic significance, and (c) explore possible mechanisms whereby segregation of integral membrane proteins may be maintained. We concentrate on epithelia, muscle tissue and neurons.

Epithelia

Functionally, epithelia form barriers between two fluid compartments and control their contents by energy-dependent transport processes. Morphologically, they are no more than a few cell layers thick and frequently only a single layer thick. At their apical margins, cells are joined together by the zonula occludens, which restricts diffusion by the paracellular route. Basally, the lateral cell membranes of adjacent cells form punctuate gap junctions which are routes of cell-to-cell communication, and desmosomes which are thought to be structural supports.

Since the principal function of epithelial is transport, it is not surprising that their cell membranes possess a variety of transport proteins, often within the same membrane (e.g. the small intestine). Knowledge of their distribution is fundamental to understanding the epithelial transport mechanisms, and, in recent years a number of studies bearing on the segregation of membrane proteins related to transport have been published. They are the subject of this section.

Sodium Chloride Transport

Three transport proteins have been identified in salt-transporting epithelia: the Na−K pump or ATPase, the amiloride-sensitive Na channel and the furosemide-sensitive Na−Cl coupled transporter. The Na−K pump is readily labeled by cardiotonic steroid inhibitors such as ouabain. The pump has been isolated, purified, and successfully

Table 1.

A. Location of Na−K pump

Epithelium	Transport direction	Location	Reference
Intestine	A → B	B	Stirling, 1972
Frog skin	A → B	B	Mills et al., 1977
Gall bladder	A → B	B	Mills et al., 1977
Urinary bladder	A → B	B	Mills & Ernst, 1975
Renal tubule	A → B	B	Shaver & Stirling, 1978
		(*)	Kyte, 1976*a*, *b*
		(♀)	Ernst, 1975
		(♀)	Ernst & Schreiber, 1981
Teleost gill	B → A	B (♀)	Karnaky et al., 1976
			Hootman & Philpott, 1979
Salivary gland	B → A	B	Bundgaard et al., 1977
Pancreas	B → A	B	Bundgaard et al., 1981
Shark salt gland	B → A	B	Karnaky et al., 1976
Avian salt gland	B → A	B (♀)	Ernst, 1972
Sweat gland	B → A	B	Quinton & Tormey, 1976
Cornea	B → A	B	*Unpublished observations*
Choroid plexus	B → A	A	Quinton et al., 1973
Retinal pigmented epithelium	B → A	A (♀)	Ostwald & Steinberg, 1980
			Unpublished observations
Trachea	B → A	B	Widdicombe et al., 1979
Ciliary (eye)	B → A	A	*Unpublished observations*
Sacculus	–	B	Burnham, 1982

Apical (A) and basolateral (B) membrane locations were determined by 3 H-ouabain autoradiography with the exceptions of: (*) immunocytochemistry, (♀) histochemistry and (♀) microdissection.

B. Location of other transport proteins

Amiloride-sensitive Na^+ channel			
Frog skin	A → B	A	Cuthbert & Shum, 1974
Urinary bladder	A → B	A	Lewis et al., 1976
Renal tubule	A → B	A	O'Neil & Boulpaep, 1979
Colon	A → B	A	Stoner, 1979
			Turnheim et al., 1978
Salivary gland	B → A	A	Schneyer, 1970
Furosemide-sensitive Cl pump			
Renal tubule	A → B	A	Burg et al., 1973
Teleost operculum	B → A	B	Karnaky (*private communication*)
			Degnan et al., 1977
Cornea	B → A	B	Ludens et al., 1980
Ciliary (eye)	B → A	A	Saito et al., 1980
Intestine	A → B	A	Frizzell et al., 1979
Phlorizin-sensitive glucose pump			
Small intestine	A → B	A	Stirling, 1967
Proximal tubule	A → B	A	Silverman, 1974

Location is based on side of antagonism and autoradiography (phlorizin only).

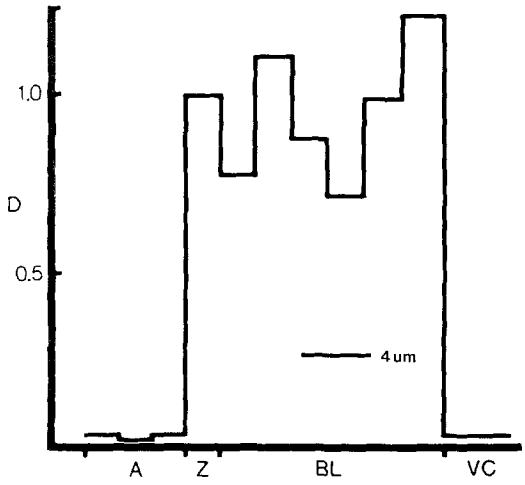
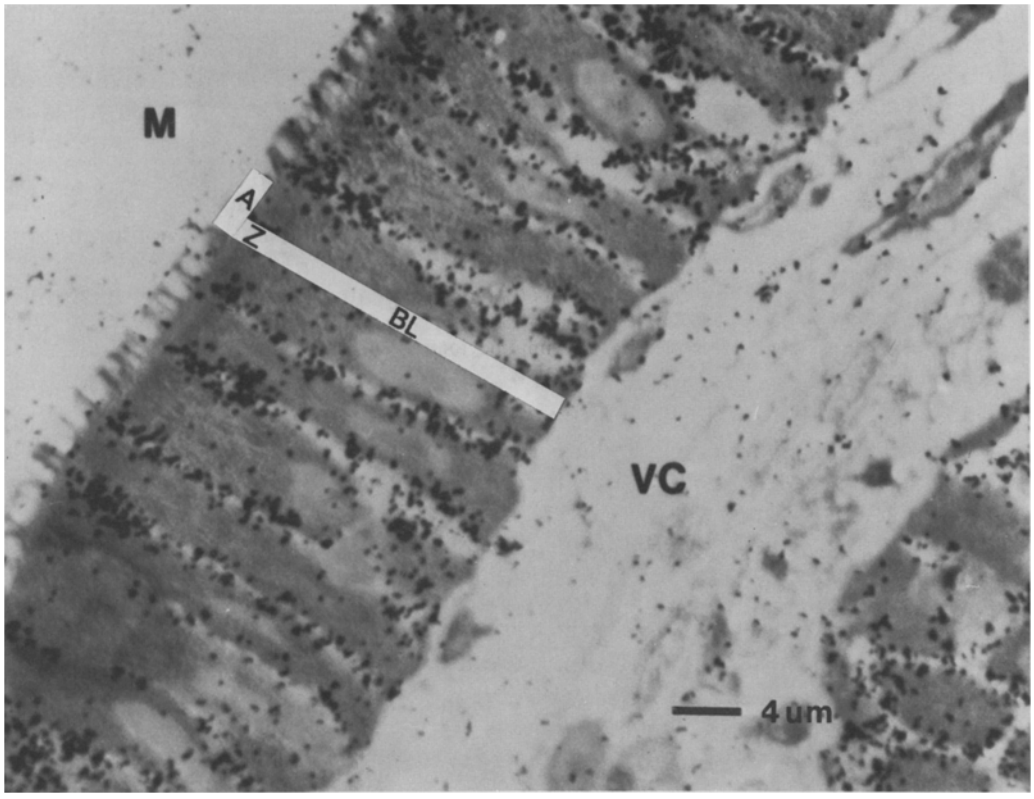


Fig. 1. The distribution of Na – K pump sites in rabbit small intestinal epithelial cells (top) was estimated from ^{3}H-ouabain autoradiographs, (bottom). The ordinate (D) gives binding or grain densities relative to the initial 2 μm of the basolateral membrane (*BL*). The abscissa gives positions along a 2-μm-wide grain counting band (bottom, shown in white). Differences in apical and basolateral membrane area/μm of cell boundary were taken into account. The spaces between microvilli of the apical membrane (*A*) are due to freezing. Other symbols are (*Z*) zonula occludens, (*M*) incubation medium, and (*VC*) villous core



reconstituted into artificial membranes and appears to be the same regardless of tissue source. The functional pump unit probably is a complex of two heavy chains and two glycosylated light chains. A more detailed discussion of this transport protein may be found in the symposium edited by Skou and Nørby (1979). It is present in all epithelia in proportion to their salt-transporting capacity regardless of whether they secrete or absorb NaCl.

The membrane distribution of this protein has been studied using a variety of techniques which are footnoted in Table 1. In all studies except one, the pump was located on either the apical or basal membrane but not both. The exception is a study by Kyte (1976*b*) in which he observed binding of ferritin-labeled antibodies (polyclonal) to both apical and basolateral membranes of rabbit proximal tubule cells. A possible explanation for this anomaly is that the antibody preparation was not entirely specific for functional Na – K pumps. It is particularly noteworthy that both histochemical and ^{3}H-ouabain autoradiographic methods suggest a very steep gradient of pump sites at the level of the zonula occludens. In Fig. 1, we have estimated the distribution of pump sites in ^{3}H-ouabain autoradiographs of small intestine (Stirling, 1972).

Clearly there are few if any apical pump or ouabain binding sites.

Figure 1 also argues against the Diamond and Bossert (1967) hypothesis that epithelia which absorb water in absence of a measurable osmotic gradient (e.g., the renal proximal tubule and small intestine) possess a descending (apical to basal) gradient of solute pumps within the basolateral membrane. The measurements indicate if a gradient of Na−K pump sites in the small intestine exists (Fig. 1), it is small. An apical-basal difference of approximately 25% would not be readily detected due to experimental error.

If Na−K pumps are freely mobile, they should be distributed randomly. This problem has received little attention perhaps because methods for visualizing specific membrane proteins are not available. Nevertheless, there is at least one epithelium in which Na−K pump sites appear to be distributed in a highly ordered fashion. Negative staining (Dendy, Philpott & Deter, 1973) and freeze-fracture (Sardet, Pisam & Maetz, 1979) studies of the teleost gill have revealed that the basolateral membrane of the chloride cell forms a fine, invaginating tubular (50 nm diam.) reticulum. Surrounding each tubule is a regular helical array of 7.5 nm particles with ~10 nm spacing. Two pieces of evidence suggest that these particles may be the Na−K pump. The 7.5 nm particle size closely agrees with freeze-fracture measurements by Maunsbach, Skriver and Jorgensen (1979) on vesicles containing purified functional Na−K pumps. More significantly a 10-nm spacing is equivalent to a density of 10^4 pump sites/μm^2. This value agrees remarkably well with a pump site density of 8.5×10^3 sites/μm^2 calculated from specific ouabain binding (240×10^{-6} mole/kg of chloride cell; Table II, in Karnaky et al., 1976) and a surface-to-volume ratio of 16.5 μm^{-1} (*unpublished measurements*). The ordered array in this case may be due to aggregation of the densely packed pump molecules. However, participation of the cytoskeleton cannot be excluded.

In most high-resistance salt-absorbing epithelia, Na enters the cell by a sodium-selective pathway (Koefoed-Johnson & Ussing, 1958; Fuchs, Larsen & Lindemann, 1977) which appears to be a channel or pore (Lindemann & Van Driessche, 1977) and is blocked by the pyrazine diuretic amiloride (Erlich & Crabbe, 1968). Amiloride is a cation and is thought to be a highly specific label for this transporter since the K_i for both inhibition and binding are in the nanomolar range, and binding is inhibited competitively by external [Na] (Cuthbert, 1981). Amiloride alters Na transport when applied to the apical side of the epithelium but not when applied to the basal side (Table 1), suggesting that these Na channels are present only in the apical membrane. To our knowledge neither autoradiographic nor biochemical (i.e., isolation of basolateral from apical membranes) localization of this transporter has been reported.

In a number of salt-absorbing and secreting epithelia, transport is inhibited by furosemide and related diuretics (Table 1). The membrane transporter affected appears to be the same as the Na−Cl cotransporter identified by Bremeshi and Henin (1975) and Frizzell, Koch and Schultz (1975). Like amiloride, furosemide is charged and does not cross cell membranes readily (*unpublished observation* on ^3H furosemide). It acts either that the basolateral or apical membrane, depending upon the epithelium; but never on both membranes (Table 1), indicating that furosemide receptors are present on but one membrane. We are unaware of published attempts to directly localize the furosemide-sensitive transporter by binding of either radiolabeled furosemide or related inhibitors. The low affinity ($K_i > 10^{-5}$ M) of these compounds is a severe obstacle in binding experiments.

An epithelium can achieve primary net transport only if the appropriate transport capacities of the apical and basolateral membranes are unequal. The above observations on NaCl transporters not only agree with this axiom but suggest that, in general, a particular transport protein is located exclusively on either the apical or basolateral membranes. The distributions of the NaCl transport proteins discussed are of major significance to the cellular mechanisms of salt transport; however, this subject, although interesting is outside the scope of this review and the interested reader is referred to papers by Ernst, Riddle and Karnaky (1980) and Frizzell, Field and Schultz (1979).

The Glucose Transporter and Hydrolases

Epithelial cells of the small intestine and renal proximal tubule actively absorb glucose and structurally related sugars by a Na-coupled mechanism. In both the intestine (Alvarado & Crane, 1962) and the kidney (Frasch et al., 1970) the plant glucoside phlorizin inhibits the Na-dependent, uphill translocation only when present in the apical fluid. Phlorizin possesses a high affinity for this transporter ($K_i \sim 10^{-6}$ M) and is not transported (Stirling, 1967); therefore, it is suitable for specifically labeling the glucose transporter in intact cells. Autoradiographic studies of hamster (Stirling, 1967)

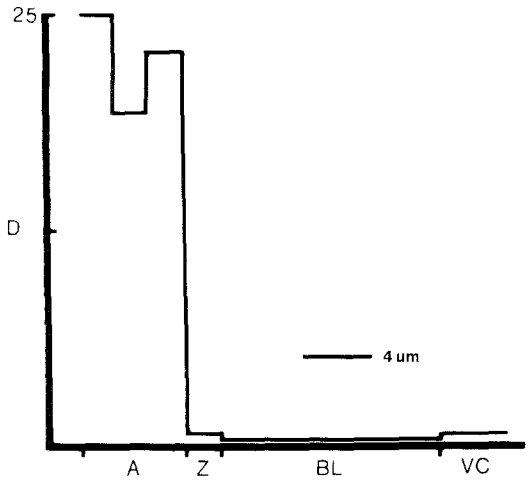
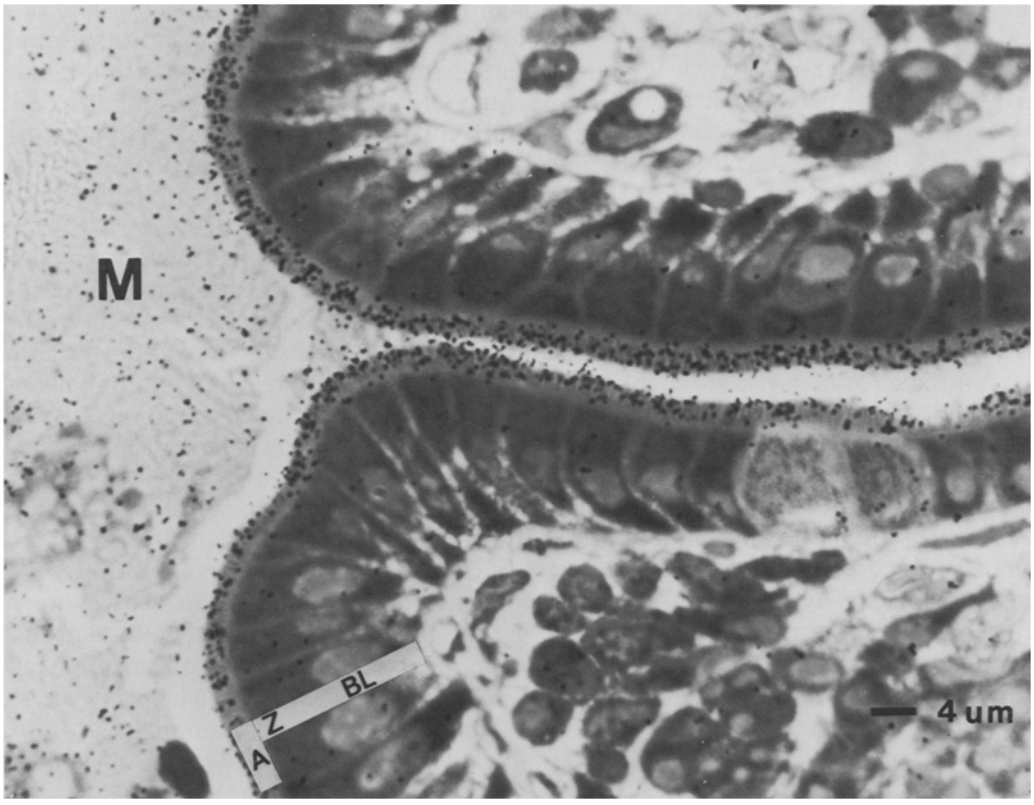


Fig. 2. The distribution of glucose transport sites in hamster small intestine (top) was estimated from ^{3}H-phlorizin autoradiographs (bottom, Stirling, 1967). The ordinate gives membrane binding site or grain densities relative to that of the incubation medium (*M*). The abscissa is as in Fig. 1



and human (Stirling, 1972) intestines indicate that the lumenal membrane binds phlorizin with a high affinity ($K_D = 1.3 \times 10^{-6}$ M and 72×10^{-6} M, respectively) and a maximal density of ~2,000 sites/μm^2 in both. These autoradiographic studies suggest a steep gradient of phlorizin binding sites at the zonula occludens. This impression is verified quantitatively in Fig. 2. Phlorizin also has been shown to bind to apical but not basolateral membranes of proximal tubule cells (Frasch et al., 1970; Silverman, 1974). Together these studies indicate that the active glucose transporter is confined to the apical membrane. Physiologically an exclusive apical membrane location is consistent with uphill cellular accumulation.

The small intestine and renal proximal tubule also possess a variety of peptidases, oligosaccharidases and phosphatases (Kenny & Maroux, 1982). Although these proteins are mostly hydrophilic (Nishi & Takesue, 1978), they may be considered integral membrane proteins since they contain a small hydrophobic moiety (5% of their mass) that

apparently spans the membrane (Louvard, Semeriva & Maroux, 1976). The hydrophobic peptide chains which form their lipid domain probably serve only as membrane anchors since they are not required for hydrolytic activity (Kenny & Maroux, 1982). These enzymes are found only in the apical membranes except in special circumstances (*Vide infra*).

Gap Junctions

Epithelial cells are coupled to each other by pathways that allow the intracellular exchange of molecules up to 2 nm in diameter (Schwarzmann et al., 1981). The structural basis for this pathway is probably the gap junction (Loewenstein, 1981) which appears as an aggregate of ~8 nm intramembranous particles in freeze-fracture images (Peracchia, 1980). It is thought that each particle consists of six subunits, and that when two such hexamers in adjacent membranes connect, they form the "*connexon*" which constitutes a cell-to-cell channel (Henderson, Eibl & Weber, 1979; Unwin & Zampighi, 1980; Baker et al., 1983). For a detailed discussion of structure-function relationships of this transport protein, the reader is referred to the informative and comprehensive review by Loewenstein (1981). It is reasonable to suppose that the *connexon* once formed, has little or no mobility because it is restrained by the membranes of two cells at once. It has been suggested that formation of *connexons* involves aggregation of gap junction precursor molecules that are scattered throughout the membrane (Loewenstein, 1981). The movement of precursor molecules towards each other may well occur by diffusion. While it is clear that the apical membrane contains no gap junctions, it cannot be ruled out that precursor molecules reside there, since precursor molecules cannot as yet be identified either histochemically or morphologically.

Gap junctions are not evenly distributed over the basolateral membrane, but congregate to form plaques. It is easy to imagine why *connexons* tend to occur in groups. Once the first *connexon* has formed and adjacent cell membranes are closely apposed, the subsequent aggregation of nearby precursors into connexons (Loewenstein, 1981) is more likely.

Neurons

Neurons conduct impulses, make chemical and sometimes electrical synapses with each other, and form at least two different kinds of junctions with glial cells. Several of these junctions involve membrane transport proteins that are highly localized. Among them are: (a) interneuronal and neuronal-glial or Schwann cell gap junctions; (b) some postsynaptic receptors for neurotransmitters and their associated ionic channels, (c) voltage-dependent ionic channels mediating impulse generation and conduction. Perhaps the most extensive studies of (a) are morphologic. This section focuses on voltage-dependent ionic channels.

Voltage-Dependent Channels: Soma vs. Axon

It is widely accepted that Ca channels are concentrated in soma and presynaptic terminals, and Na channels in the axon. Katz and Miledi (1969) observed that presynaptic nerve terminals at the squid giant synapse are capable of electric excitation that is resistant to tetrodotoxin and dependent on external Ca. Evidently excitation is due to voltage-dependent Ca channels. Only those portions of the terminals within 1 mm of the synaptic region contribute to the response; the squid axon itself is apparently inexcitable in the presence of tetrodotoxin. The nerve cell soma is also capable of regenerative Ca action potentials, at least in snails (Meves, 1968). Ca currents under voltage clamp are readily recorded from presynaptic terminals of the squid giant synapse (Llinás, Steinberg & Walton, 1976) and snail neuron soma (for a review *see* Hagiwara & Byerly, 1981). The physiologic purpose of Ca channels is to link electric excitation to synaptic transmitter release and, possibly, to changes of cell (soma) metabolism.

With regard to Na channels, it has been known for some time that, at least in vertebrate neurons, the nerve impulse originates not in the soma but at the axon hillock, the point where the axon emerges from the soma (e.g., Coombs, Curtis & Eccles, 1957; Fuortes, Frank & Becker, 1957). This "initial segment" of axon is not myelinated even if the remainder of the axon is. Computer simulations by Dodge and Cooley (1973) suggest that in vertebrate motoneurons, Na-channel density in the inital segment may be as high as that of the node of Ranvier (*see later*), and declines steeply towards the nerve cell body and dendrites.

Catterall (1981) has studied the distribution of Na-channels in cultured spinal cord neurons by autoradiography, using an iodinated scorpion toxin as a ligand. He found a sevenfold higher activity over the neurites than over the cell body. Proceeding from cell body to neurite, the grain density changed from low to high within 10 μm.

In an attempt to localize ionic channels on voltage-clamped *Aplysia* nerve cell bodies, Kado (1973) explored the cell surface with extracellular micropipettes. Tetrodotoxin-sensitive (presumably Na) currents were largest at the point where the axon emerged from the soma, whereas toxin-insensitive (presumably Ca) currents were smallest and increased with distance from that point. Outward currents (probably through voltage or Ca-activated K channels) also increased with distance from the axon. Kado's (1973) experiments provide information only on the *relative* distribution of Na and Ca channels. In absolute terms, the density of Na channels may exceed that of Ca channels even at the pole of the neuron opposite to that where the axon originates.

Myelinated Nerve: Node of Ranvier vs. Internode

The myelin sheath of axons is periodically interrupted, and leaves 1 to 2-μm-wide gaps where the axon is directly exposed to the endoneural space. These small gaps or "nodes of Ranvier" are the sites of the electrical excitation. The node contains approximately 100 μm^2 of axolemma with 10^5 to 10^6 Na channels (frog node: 2,000/μm^2, Conti et al., 1976; rabbit node: 12,000/μm^2, Ritchie & Rogart, 1977) as well as many Na–K pump sites. Ritchie and Rogart (1977) suggested that Na channels may be confined to the node of Ranvier. This view was supported in a later paper by Chiu and Ritchie (1980). Normal rabbit nodes of Ranvier under voltage clamp show large Na currents, but only small (or no) K currents, consistent with the view that the nodal density of Na channels is much larger than that of K channels. After treatment with collagenase followed by mild osmotic shock, conditions which apparently "loosen" or "lift off" the myelin sheath in the paranodal region, Chiu and Ritchie (1980, 1981) observed a sudden and large increase in membrane capacity, accompanied by an equally sudden appearance of large outward currents with the pharmacologic and kinetic properties of K channels. They ascribed these effects to a sudden detachment of myelin from the paranodal region. This would allow their voltage clamp to collect current from a much larger area of axonal membrane, hence the sudden increase of capacity. Apparently, the newly uncovered membrane contains numerous K channels. By contrast, the magnitude of Na currents did not change, thus the paranodal axolemma contains few or no Na currents.

Following Chiu and Ritchie (1980), a rough estimate of the population densities of Na and K channels can be made. In their Fig. 1, paranodal demyelination produced an increase in membrane capacity from 2.5 to 35 pF but maximal Na-current stayed constant, increasing from 14.5 nA by at most 5%. Therefore, the current relative to membrane capacity was $14.5/2.5 = 5.8$ nA/pF before demyelination and any newly appearing Na current (< 0.7 nA) was accompanied by a 32.5 pF increase in membrane capacity. If Na current from the nodal region did not change during demyelination, if membrane capacities of nodal and internodal membranes are equal and if problems of voltage control in the newly uncovered paranodal region may be ignored, it follows that the average Na current in the rabbit paranodal axolemma is less than 24 pA/pF, and the Na channel density therefore 250 times less than at the node of Ranvier.

Since the node of Ranvier consists of a narrow (< 2 μm wide) and sharply defined ring of axolemma, the channel density must vary extremely steeply between nodal and paranodal axolemma. If channel density in the nodal area is uniform and vanishes exponentially with distance from the nodal area, then it must fall *e*-fold in less than 50 nm in order to keep the contribution of extranodal channels below 5%. If the Na channel density does not fall to zero (say, 1 to 2%) then the gradients are steeper.

Differences between nodal and inter- or paranodal axolemma have been observed also by freeze-fracture. The extracellular leaflet (E-face) contains large intramembrane particles (IMP) at a density of 1,200/μm^2 (Rosenbluth, 1976; frog brain) or 400/μm^2 (cat spinal cord, Kristol, Sandri & Akert, 1978); these particles occur in the internodal sarcolemma at a much lower density (4/μm^2, Kristol et al. 1978). In the E-face, smaller particles also occur at higher density in nodal than in internodal axolemma. Overall particle densities in nodal and internodal E-faces are given as 1,200 to 1,300/μm^2 and 100 to 200/μm^2, respectively, in cat spinal cord (Kristol et al., 1978) as well as in peripheral nerve of frogs and tadpoles (Tao-Cheng & Rosenbluth, 1980). In the cytoplasmic membrane leaflet (P-face), particle densities of both nodal and internodal sarcolemma are similar, namely 1,200 to 1,600 in cat spinal cord and frog peripheral nerve. It is widely believed that the large particles of the E-face are Na channels. Consistent with this view, they are found in the electric organ of the knifefish *Sternarchus albifrons* only at electrically excitable, but not at inexcitable nodes of Ran-

vier (Kristol et al., 1978). However, it has also been pointed out that the observed densities of large particles are less than the sodium channel densities inferred from electrical measurements. In addition to several factors that have been invoked to explain this discrepancy (Kristol et al., 1978; Tao-Cheng & Rosenbluth, 1980), we suggest as an additional possibility that sodium channels may occur as oligomers. This is supported by a comparison of the number of binding sites for saxitoxin and a scorpion toxin, two specific ligands for the sodium channels. In neuroblastoma cells (Catterall & Morrow, 1978) as well as in frog skeletal muscle (Almers & Levinson, 1975; Catterall, 1979), there appear to be two to three times more binding sites for saxitoxin or tetrodotoxin than there are for scorpion toxin. If saxitoxin and tetrodotoxin bind Na channels with 1:1 stoichiometry, as generally believed, and if scorpion toxin can modify most sodium channels, then a single scorpion toxin molecule must be able to modify more than one Na channel. This suggests an extremely close proximity between channels. Furthermore, single-channel recordings have shown that in small, electrically isolated patches of neuroblastoma cells the number of sodium channels encountered is usually even (Aldrich, Corey & Stevens, *personal communication*). Oligomerism would explain why (a) the particles are so large and (b) the density is higher in electrical than in morphologic measurements. Regardless of whether or not the large particles are sodium channels, it is clear that their density changes abruptly from node to paranode (within 100 nm in Fig. 3 of Tao-Cheng & Rosenbluth, 1980). Therefore, both electrophysiological and morphological measurements indicate extremely steep concentration gradients for membrane proteins at the node of Ranvier. Immunohistochemical studies on myelinated nerve of electric eel are consistent with Na channels residing almost exclusively at the node of Ranvier (Ellisman & Levinson, 1982). Similar studies also suggest that sodium pumps (Na−K-ATPase) are crowded together at high density in the nodal axolemma (Wood et al., 1977).

Potassium channels appear less discretely localized. Chiu and Ritchie (1981) found an average outward K-current of 4.9 nA before and 34.7 nA after demyelination in fibers where capacity increased from 1.4 to 86 pF. Thus, the average current densities are 0.4 nA/pF in the paranodal, and 3.5 nA/pF in the nodal region. Nodal density may be higher than paranodal, but the difference is less than with Na current. Also, because some of the K current in normal nodes may not flow through conventional voltage-dependent K channels (Chiu & Ritchie, 1980) and because "normal" nodes may already have suffered some small demyelination of paranodal axolemma during the stresses applied to the fibers during dissection, the I_K-density of the normal node is not accurately known. In a more recent paper on bullfrog myelinated nerve (Chiu & Ritchie, 1982), K channels were also seen in the internodal axolemma after the myelin was removed with lysolecithin. Under 150 mV depolarization, K currents there have a density of ~2 nA/pF, as compared to ~20 nA/pF at the frog node of Ranvier (*Rana pipiens*; Stampfli & Hille, 1976). The physiologic function of internodal and paranodal K channels is unclear.

Photoreceptors

Vertebrate photoreceptors are morphologically divided into two segments that are tenuously connected by a narrow stalk, the ciliary junction. The outer segment contains abundant visual pigment, rhodopsin, along with the molecular machinery for transforming light into electrical signals. The proximal segment contains the nucleus and makes synapses with other neurons of the retina. The outer segment is continually renewed at the ciliary junction and phagocytized distally by the retinal epithelium (Young, 1967). A steady current flows between the two segments; it is outward across the cell membrane of the inner, and inward across the membrane of the outer segment. The current is generated by a Na−K pump located exclusively in the proximal segments (Bok & Filerman, 1979; Stirling & Lee, 1980) and flows through a Na permeable, light-sensitive channel located (perhaps exclusively) in the outer segment. The density of Na−K pump sites in the inner segment is unusually high (3.4×10^3 sites/μm^2; Stirling, *unpublished*) and that of the outer segment at least 100 times less, with the transition from high to low occurring within less than 10 μm (Stirling & Lee, 1980; ^{3}H-ouabain autoradiography). The cell membranes of the outer segment and cilium contain an abundance of apparently freely mobile visual pigment (Wei, Cone & Edidin, 1981), while the membrane of the inner segment contains little or none (for the most recent demonstration of this *see* an immunocytochemical study by Peters et al., 1983). Thus photoreceptors maintain a reciprocal segregation of at least two cell membrane proteins: rhodopsin and Na−K pumps. Peters et al. (1983) have shown that rhodopsin is inserted into the cell membrane in the small region of inner segment immediately next to the cilium. By unknown mech-

anisms, it is prevented from spreading over the remainder of the inner segment, and instead is directed into the outer segment. The turnover time for disk membranes and presumably rhodopsin is of the order of 6 weeks (Young, 1967). How this cell achieves and maintains segregation of an (ultimately) freely mobile rhodopsin is still unclear.

Muscle

The cell membrane of vertebrate skeletal muscle has two components, the sarcolemma or surface membrane, and a system of invaginations called the transverse tubular system (TTS). Distribution of membrane transport proteins over the sarcolemma is discussed first; then an account is given of the distribution between sarcolemma and TTS.

ACh Receptors (AChR)

The myoneural junction has been extensively studied as a prototype for chemical synapses. Autoradiographic studies (e.g. Fertuck & Salpeter, 1976) have shown that AChR are strictly confined to the "motor endplate" sarcolemma and form arrays that are sufficiently dense at the crests of the junctional folds (21,000/μm^2) that adjacent AChR nearly touch each other. At the edge of the arrays, the AChR density falls to a few percent within <1 μm (Fertuck & Salpeter, 1976). Given the resolution limits of autoradiography, the gradients could be even steeper. Similarly focal localization of postsynaptic receptors also may occur in neurons (*see*, e.g., Gulley & Reese, 1981).

There are indications that the cell membrane at the endplate may be specialized in other ways. For example, the endplate acts as a "current sink" (Betz et al., 1980) to the rest of the muscle. Werman (1960) reported that the focally recorded inward current during a propagated muscle action potential is less at the endplate than elsewhere, possibly suggesting a lower local density of Na channels. The presynaptic axolemma at the "motor end plate" also exhibits a high degree of organization. Freeze-fracture studies (*see* review by Ceccarelli & Hurlbut, 1980) have revealed a double row of large (~ 120 nm) intramembrane particles on either side of the membrane ridge which borders the "active zone" or region of vesicle attachment. The function of these particles is not known; however, their close spatial relationship to the "active zone" vesicles is suggestive of voltage-dependent Ca channels.

Sodium and Potassium Channels

When the surface of frog skeletal muscle fibers is explored with extracellular glass micropipettes under patch voltage clamp conditions, Na and K currents are found to vary with location on the muscle fiber (Almers, Stanfield & Stühmer, 1983), probably because the concentration of Na and K channels varies in the plane of the membrane. The concentration of Na channels may vary twofold within less than 20 μm. Sodium channels seem to be congregated in strips or oblong patches running along a fiber. The distribution of K channels is irregular. Also, no correlation between spots of increased channel density and other structures have been found. The physiologic significance of these "hot spots" is not understood.

Sarcolemma vs. Transverse Tubular System (TTS)

Several membrane transport proteins are unevenly distributed between TTS and sarcolemma. Distribution has been studied with two classes of experiments. In the first, the continuity between sarcolemma and TTS is disrupted by sudden withdrawal of glycerol from glycerol-loaded fibers. Since after glycerol treatment, the sarcolemma reseals but remains isolated from the TTS, any electrical or toxin binding property that is missing from glycerol-treated muscle fibers can be attributed to the TTS. The second method makes use of the fact that ion movements across the tubular membrane will cause ion accumulation or depletion in the tubule lumen. Electrophysiologic consequences of ion accumulation or depletion can then be analyzed, and any fraction of ion permeability that is modulated by concentration changes is assigned to the TTS. Table 2 shows relative densities of a number of ion transport proteins, as calculated by Almers, Fink and Sherpherd (1982). The experiments forming the basis of Table 2 are now discussed.

Na Channels

From the difference in saturable tetrodotoxin binding before and after glycerol treatment, Jaimovitch, Venosa, Shrager and Horowicz (1976) calculated that in a 55 μm diameter fiber, about 54% of the sodium channels reside in the TTS. Considering the relative contributions of TTS and sarcolemma to total cell membrane area, it can be calcu-

Table 2. Distribution of membrane transport sites between sarcolemma and transverse tubular systems of frog skeletal muscle

Channel	Fractions in TTS (%)[a]	Density TTS/ Sarcolemma[a]	Average fiber diameter (μm)[a]
Na^+ channel[b]	55	0.7	55
Delayed K^+ channel[c]	50	0.3	~90
Inward rectifier channel[d]	75–80	~1	80–120
Cl^- channel (rat)[e]	≧60	~1	~60
Ca^{++} channel[f]	93–100	>4	97
Na/K ATPase[g]	~20	0.15	52

[a] Fractions in TTS and average fiber diameter as given in the references quoted. Density TTS/sarcolemma was calculated by assuming that the ratio of tubular to sarcolemmal area is given by 647 a/cm where a is the fiber radius (Almers et al., 1982).
[b] Jaimovitch et al., 1976.
[c] Kirsch et al., 1978.
[d] Almers, 1972; Schneider & Chandler, 1976.
[e] Palade & Barchi, 1977.
[f] Almers et al., 1981.
[g] Venosa & Horowicz, 1981.

lated that the average density of the Na channels in the TTS is about half that of the sarcolemma. It has been suggested (Almers et al., 1982) that the transition between sarcolemmal and tubular Na channel densities is gradual, and that tubular Na channel density declines radially, with the central portion of the TTS having the lowest density. Such a profile is expected if sodium channels were inserted into the sarcolemma, then invaded the TTS by diffusion within the membrane, and survived there for only a finite time. Based on the difference in densities, quantitative treatment of this idea (Almers et al., 1982; *see later*) suggests that a sodium channel may diffuse an average radial distance of ~10 μm before becoming nonfunctional. This characteristic distance, termed λ, also is a measure of how steeply the density changes with distance.

Voltage-Dependent K Channels

Muscle action potentials are followed by a slow after-depolarization that is probably the result of K accumulation in the TTS. From the K efflux during an action potential and the amplitude of the after-depolarization, Kirsch, Nichols and Nakajima (1978) calculated that in a 90 μm diameter fiber, about 50% of the voltage-dependent ("delayed-rectifier") K channels reside in the TTS. If so, the average density in the TTS is about 0.4 times that in the sarcolemma, and the maximal characteristic diffusion distance of K channels in the TTS (calculated as in Almers et al., 1982) is 6 μm or less.

Ca Channels

Glycerol treatment causes loss of Ca channels along with a roughly proportional decrease in cell membrane area as judged by membrane capacity measurements (Nicola-Siri, Sanchez & Stefani, 1980). This suggests that most Ca channels reside in the TTS. Furthermore, Ca current into EGTA-loaded fibers declines with time when the sarcolemma is subjected to sustained depolarization by a voltage clamp. This decline is probably due to Ca depletion from the TTS (Almers, Fink & Palade, 1981). Analysis of depletion transients suggests that in a 97 μm diameter fiber, more than 93% of all Ca channels reside in the TTS. Considering membrane areas, the density of Ca channels in the TTS must be more than three times as high as that in the sarcolemma.

Na–K Pump

Venosa and Horowicz (1981) found that saturable binding of tritiated ouabain is diminished by only 20% after glycerol treatment. Since the average fiber diameter in their experiments was probably about 54 μm, the TTS to sarcolemma membrane area ratio was about 3:1 and the average sarcolemmal density of Na–K pump sites was at least 15 times higher than that of the TTS (*see also* Narahara et al., 1979). There is evidence suggesting that the tubular membranes contain at least some pump sites, since membrane vesicles of tubular origin exhibit ouabain-sensitive sodium uptake (Lau, Caswell, Garcia & Letellies, 1979; Narahara et al., 1979). Furthermore, after mechanical removal of the sarcolemma, certain contractile events in rabbit muscle remain ouabain-sensitive (Donaldson, 1982). The observations of Venosa and Horowicz (1981) suggest that the Na–K pump site gradient between sarcolemma and TTS is steep. It can be calculated that sodium pump sites are allowed a characteristic diffusion distance or less than 1 μm.

Other Transport Proteins

Data are available also on the two ionic channels responsible for the resting potential: Cl channels and a second type of K channel with a unique voltage dependence, termed "inward rectifier." A variety of different experiments (reviewed by Almers et al., 1982) has shown that the inward rectifier populates sarcolemma and tubular mem-

branes at about equal density. With Cl channels, the situation is less clear. In rat skeletal muscle, Palade and Barchi (1977) reported a substantial loss of Cl conductance after glycerol treatment, consistent with approximately equal density in TTS and sarcolemma. In frog muscle, glycerol treatment reportedly causes no loss in Cl conductance. However, there is evidence for at least some chloride permeable channels in the transverse tubules of frog muscle (Almers et al., 1982).

The muscle cell membrane must contain other proteins involved in solute transport, such as the Ca pump and proteins involved in uptake of sugars and amino acids, as well as the Ca-activated K channels found in tubular membrane fractions. There are as yet not data concerning distribution of these membrane proteins. Also, the transverse tubular membrane presumably contains the structures that control calcium release from the sarcoplasmic reticulum. The electron-dense structures forming the junction between TTS and sarcoplasmic reticulum (Franzini-Armstrong, 1970) may be involved in control of Ca release. Charge movements are thought to signal an early event in this process (Schneider & Chandler, 1973; Almers, 1978).

Physiologic Considerations

It seems that ionic channels involved in maintenance of the resting potential (K, Cl channels) are evenly distributed over tubular and sarcolemmal membranes. Those involved in excitation (voltage-dependent K, Na channels) are more numerous on the sarcolemma. Perhaps large ionic currents across the sarcolemma are necessary for achieving an adequate velocity for impulse conduction along muscle fibers that are several centimeters long. On the other hand, conduction into the TTS occurs over distances of a few tens of micrometers, so high velocities are not required. Moreover, too many K channels in the TTS could lead to excessive K accumulation which would cause repetitive discharges as observed in myotonic goats (Adrian & Bryant, 1974). Having too many sodium channels in the TTS could also lead to an unstable membrane potential. With Na−K pumps, it could be disadvantageous to have them located predominantly in membranes bordering on a small extracellular space. On the other hand, the physiologic role of Ca channels in the TTS is unclear.

Segregation and Mobility

Table 3 summarizes the examples of nerve, epithelium and muscle discussed so far. In all cases, steep concentration gradients are a permanent feature of adult tissue. For epithelia, few quantitative estimates of gradients are available (Figs. 1 and 2), but it is likely that steep gradients across the tight junctions exist for all of the proteins cited. In several instances the characteristic distance λ (*see* p. 178 or legend of Table 3) has values in the micron or submicron range. Any such gradient will be opposed by thermal agitation, as molecules tend to diffuse away from where the cell would like to keep them. For segregation to be maintained, the membrane protein must either be immobilized (e.g., by binding to immobile cell structures) or there must be an organizing force that continually sorts and retrieves molecules that have diffused away.

Electric Fields

This force could be electrical, as suggested by the findings of Poo and collaborators (Poo, 1981) that integral membrane proteins may redistribute under weak electric fields in the plane of the membrane. Concanavalin-A receptors on spherical *Xenopus* myotomal cells are initially distributed uniformly, but under an extracellular field of only 1 mV/μm, they accumulate at the cathodal side of the cell within ~30 min. The cathodal/anodal concentration ratio may grow to 2 on cells of 35 μm diameter (Poo, Lam, Orida & Chao, 1979). Salt-transporting epithelia, as well as the retinal photoreceptor layer, maintain a steady potential across them. In epithelia, extracellular fields vary in polarity and are between 0 and 50 mV/μm, depending on the epithelium and on whether the potential drops entirely across the tight junction or over a distance corresponding to the thickness of the epithelial cell layer. The extracellular field across the photoreceptor layer is only about 10 μV/μm, outer segment positive (Hagins, Robinson & Yoshikami, 1975), but the field is due to large currents that could cause a much larger intracellular electric field, especially along the cilium. Though there is little detailed knowledge about extra- or intracellular fields in vertebrate tissues it seems as though these fields could in some cases be large enough to influence the distribution of membrane proteins. Segregation by electric fields ("electrophoresis") would be an attractive mechanism to segregate membrane proteins that require free mobility to be physiologically active. For example, it is thought that hormone receptors must interact with other integral membrane proteins in order to stimulate the production of a second messenger. Possibly, a steady cytoplasmic or extracellular electric field could act to confine the visual pigment rhodopsin to the outer segment of photoreceptors.

Table 3. Lateral gradients in membrane protein concentration

Protein	Preparation	Gradient (high-low)	λ[a] (μm)	References
Neurons				
Ca channel	Squid giant synapse	Terminal-axon	<1,000	Katz & Miledi, 1969
Ca channel	*Aplysia* neuron	Soma-axon	?	Kado, 1973
Na channel	*Aplysia* neuron	Axon-soma	?	Kado, 1973
Na channel	Vertebrate neurons	Axon-soma	?	Dodge & Cooley, 1973
Na channel	Cultured spinal neurons	Axon-soma	<10	Catterall, 1981
Na channel	Node of Ranvier	Node-internode	<0.05	Chiu & Ritchie, 1981
Na−K pump	Retinal photoreceptors	Inner-outer segment	<5	Stirling & Lee, 1980
Rhodopsin	Retinal photoreceptors	Outer-inner segment	<10	Peters et al., 1983
Epithelia				
Na−K pump	Rabbit intestine	Basolateral-apical	<3	Fig. 1
Glucose transporter	Hamster intestine	Apical-basolateral	<3	Fig. 2
Vertebrate skeletal muscle				
Ca channels	Frog	Tubules-sarcolemma	?	Almers et al., 1981
Na channels	Frog	Sarcolemma-tubules	<10[b]	Jaimovitch et al., 1976; Almers et al., 1982
Na channels	Frog	"Hot spots" on sarcolemma	<10	Almers et al., 1983
Delayed K^+ channels	Frog	Sarcolemma-tubules	<6[b]	Kirsch et al., 1978
Na/K ATPase	Frog	Sarcolemma-tubules	<1	Venosa & Horowicz, 1981
ACh receptor	Frog	Myoneural junction	<0.3	Fertuck & Salpeter, 1976
ACh receptor	Frog	Away from tendon end	~70	Katz & Miledi, 1964

[a] Throughout, λ is an estimate of the distance over which local concentration of membrane protein falls *e*-fold.
[b] Estimate of average radial diffusion distance before failure, as calculated in Almers et al., 1982.

There is as yet no clear evidence that electric fields are used physiologically to maintain permanent (or long-term) segregation of membrane proteins. Segregation by electrophoresis as a general mechanism has some disadvantages. (1) Sorting by electric charge and mobility may lack specificity. (2) With ion transport proteins, e.g., in the retina or epithelia, electric fields could be the consequence rather than the cause of segregation. (3) In many instances (AChR at the myoneural junction, Na channels in frog sarcolemma) steep gradients seem to persist in absence of significant electric fields. This also appears to be true for epithelia and photoreceptors, since long exposures (>30 min) to supermaximal inhibitory concentrations of ouabain did not alter Na−K pump gradients (Stirling & Lee, 1980).

Immobilization

Immobilization is definitely used by some cells to maintain concentration gradients. Junctional AChR (Axelrod et al., 1976) have a mobility that is at least several thousand times less than expected for an unrestrained solitary protein in a fluid lipid bilayer. Even after extraction of the lipid bilayer by detergents, AChR aggregates in embryonic muscle cells remain associated with the cell (Prives et al., 1979), indicating that they are attached to constituents that are soluble neither in water nor detergent. Deep-etch freeze-fracture experiments have revealed that postsynaptic receptors at an inhibitory central synapse (Gulley & Reese, 1981) and AChR (Hirokawa & Heuser, 1982) are closely associated with, or even attached to, the "postsynaptic densities" that form an intracellular electron-dense coating of the postsynaptic membrane. Other membrane transport proteins that are known to be essentially immobile are Na channels in frog sarcolemma (Stühmer & Almers, 1982) and most membrane protein of intact erythrocytes (predominantly band-3, Peters et al., 1974).

It is of interest to know whether the mobilities of the other transport proteins in Table 3 are similarly restricted. The observed steep concentration gradients suggests that this might be so. In order to relate mobility and concentration gradients, assume that once proteins have been inserted into the cell membrane, they experience no displacement forces other than thermal agitation. Consider that in a specific region of the cell membrane, the "source region," the concentration of a given protein, P, is maintained high and constant by metabolic processes, such as insertion of new molecules

into the cell membrane. As molecules diffuse away from the source, they will eventually become nonfunctional and internalized. Therefore, the concentration of P will diminish with distance from the source. In the steady state, the steepness of concentration gradients then depends on the "mean diffusion distance before failure," that is, the average distance a P-molecule can diffuse before becoming nonfunctional. This distance can be shown to be (Almers et al., 1983):

$$\lambda = (TD)1/2 \quad (1)$$

where T is the mean survival time and D the diffusion coefficient. For unidimensional diffusion away from the source, i.e., diffusion from the nodal into the paranodal axolemma, the steady-state concentration of P as function of distance (x) from the source (o) is given by:

$$P_x = P_o \exp(-x/\lambda). \quad (2)$$

Other cases of interest are diffusion away from a circular spot (e.g., postsynaptic receptors diffusing away from the synapse) or diffusion from the perimeter into the center of the circle (e.g., diffusion of Na channels from the sarcolemma into the transverse tubular system). These geometries are considered elsewhere (Almers et al., 1982, 1983).

The values for λ given in Table 3 are variable, but several of them are of the order of a few μm or below. If the mobility of solitary copies of P were limited entirely by the viscosity of the lipid bilayer, one would expect values of at least $D = 2$ to 4×10^{-9} cm^2/sec, as has been measured for rhodopsin in rod outer segment disks (4×10^{-9} cm^2/sec; Poo & Cone, 1974). With λ in the micron range, the cells in Table 3 would have to turn over the proteins in question within minutes or tens of minutes. Such turnover rates would be unusually high. Functional AChR in rat diaphragm, for instance, have a life expectancy of about 23 days (Bevan & Steinbach, 1983). With $\lambda < 1$ μm, one expects $D < 10^{-14}$ cm^2/sec, well below the limit of resolution in current photobleaching experiments. Other membrane proteins are similarly long-lived. For most integral membrane proteins in hepatoma liver cell line (Tweto & Doyle, 1976), the survival time is about 100 hr. For rhodopsin, it is probably about equal to the turnover time of rod outer segments, or ~42 days. For transport proteins in erythrocyte membranes, it must be of the order of months, as erythrocytes survive 120 days without protein synthesis. Renaud, Romey, Lombat and Lazdunski (1981) found that aggregated cardiac muscle cells in tissue culture lose fewer than 5% of their sodium channels within 24 hr after blockage of protein synthesis; this suggests that the survival time of sodium channels may be greater than 1.5 weeks in that tissue. With a survival time of one week and a concentration gradient corresponding to $\lambda = 10$ μm, the lateral mobility of a membrane protein would have to be of the order of only $D = 3 \times 10^{-12}$ cm^2/sec. In conclusion, most of the membrane proteins in Table 3 are likely to have severely restricted mobilities. Anchoring of membrane transport proteins may be fairly common.

Possible Mechanisms for Restricting Lateral Mobility

Aggregation of AChR

In embryonic *Xenopus* muscle ("myotomal") cells, most AChR are diffusely distributed over the cell membrane, and appear freely mobile (Poo, 1981). However, once they have been concentrated on one side of the cell by a steady electric field, they remain there indefinitely even after the field is turned off (Orida & Poo, 1978). Evidently, AChR aggregate if they are crowded together. These aggregates are as stable as naturally occurring AChR aggregates, and are resistant to various drugs generally believed to disrupt cytoskeletal structures. However, extracellular trypsin apparently disperses both natural and field-induced aggregates (Orida & Poo, 1980). In Orida and Poo's experiments, aggregated AChR were apparently held together by bonds between peptides that are "extracellular" or, more precisely, accessible to membrane-impermeant external enzymes. Within aggregates of AChR, individual AChR's are immobile, at least in rat embryonic muscle (Axelrod et al., 1976). This tendency of AChR's to aggregate may well be important to the stability of the neuromuscular junction. However, anchoring to intracellular structures probably also plays a role. Another clear case of immobilization by aggregation is the two-dimensional crystals of bacterial rhodopsin found in the purple patch of *Halobacterium halobium* (e.g., Blaurock & Stoeckenius, 1971).

Relationship to Cytoskeleton

Extensive cytoskeletal structures are often found subjacent to cell membranes (or cell membrane regions) that contain highly localized or immobile integral membrane proteins. The membrane of erythrocytes is internally coated with spectrin, a tetrameric protein with an aggregate molecular

weight of 900,000 daltons (Marchesi, 1979). "Postsynaptic densities" (Palay, 1958) containing a unique 50,000-dalton protein termed "PSD-50," plus actin, tubulin, and other proteins, are regularly found beneath postjunctional membrane regions. In myelinated nerve, there are two discrete regions of axolemma containing a high density of Na channels, namely the initial segment and the node of Ranvier; both regions contain an electron-dense undercoating that stains intensely with ferric ion or ferrocyanide. The undercoating is absent from the internodal axolemma which does not contain Na channels (*see* Waxmann & Quick, 1978, for a review).

Spectrin, postsynaptic densities and ferrocyanide-binding material could all be cytoskeletal anchors for membrane proteins. However, it is unknown or even questionable whether these materials bind directly to ion transport proteins. Instead, the connection may be made via linkage peptides that bind specifically to some (or one) kind of membrane proteins and not to others. In erythrocytes, the linkage between band-3 protein and spectrin is thought to be made via a 200,000-dalton protein, ankyrin (Bennett & Stenbuck, 1979*a*). Unlike band-3, ankyrin is not a transmembrane protein; however, ankyrin is released from membranes only after treatment with detergent (Bennett- & Stenbuck, 1979b). In *Torpedo* electroplaques, AChR's include an intracellular 43,000-dalton subunit peptide that is probably not necessary for transmitter-induced ion transport by the AChR (e.g. Heidmann, Sobel, Popot & Changeux, 1980) and can be dissociated from the AChR by alkaline treatment. Also lost by such treatment is a characteristic electron-dense membrane coat normally observed in vesicular preparations of native AChR (Cartaud et al., 1981). The membrane coat is probably derived from the postsynaptic density, and it has been suggested that the 43,000-dalton subunit serves to link AChR's to each other and/or to the postsynaptic density.

The mechanisms whereby membrane proteins are linked to cytoskeletal components are yet to be completely elucidated. One may expect that at least one element in the linkage shows a specific affinity to the membrane protein whose mobility the cell wishes to restrict. Immobilization of cell membrane proteins may be reversible, in that there may be an equilibrium between two pools for a given protein: one freely mobile, the other bound to some anchoring site. For several of the proteins in Table 3, the equlibrium must be strongly in favor of the anchored state, as the density of membrane proteins over the cell membrane can vary over many orders of magnitude.

Is the Cell Membrane Divided into Compartments?

Segregation by barriers has been proposed for two cases. In epithelia, the zonula occludens (tight junction) was thought to separate apical and basolateral membrane proteins (Pisam & Ripoche, 1976), and in myelinated nerves, the axoglial junction between axon and its glia or Schwann cell are thought to confine sodium channels to the node of Ranvier (Rosenbluth, 1976). Both issues are now discussed.

At the node of Ranvier, newer evidence indicates that sodium channels and other node-specific particles are probably held by other than mechanical constraints. In "dystrophic" mice of the 129/Rej-Dy strain, dorsal and ventral roots contain axons that are only partially myelinated. At these incompletely myelinated sections or "heminodes," the large particles of the E-face continue to congregate at high density, even though on one side, there is no Schwann cell to serve as a barrier (Ellisman, 1979). In developing nodes, the characteristic ferrocyanide-binding axolemmal undercoating is confined to the region of a future node already before arrival of the longitudinally growing myelin sheath (Waxman & Foster, 1980). If the ferrocyanide-binding material marks the location of sodium channels, then the congregation of these channels cannot be caused or maintained by myelin. More likely, the axolemmal undercoating serves as a cytoskeletal anchor for sodium channels (Ellisman, 1979; Bray, Rasminski & Aguayo, 1981). In skeletal muscle, sarcolemmal sodium channels are immobile (Stühmer & Almers, 1982) and congregate in patches (Almers, Stanfield & Stühmer, 1983) in absence of extracellular specializations such as myelin.

In epithelial cells, the view of the zonula occludens as a barrier was suggested by the observation that cell membrane proteins intermix after the zonula occludens is disrupted. Pisam and Ripoche (1976) labeled components of frog bladder apical membrane by [^{3}H] sugar incorporation, ^{125}I iodination, concanavalin-A peroxidase, and other histochemical techniques, and followed the distribution of label after disrupting the zonula occludens. After $\sim$5 min, labeled material previously confined to the apical border appeared in the opened junction and progressively invaded the cell membrane. After 80 min, the now-spherical cells

were uniformly labeled. Meldolesi et al. (1978) examined acinar cells of the guinea pig pancreas by freeze-fracture. They found the intramembrane particle density of the lumenal (apical) membrane to be less than one-half of that of the basolateral membrane in the intact epithelium. With minutes after disruption of the tight junctional complex by incubation in Ca-free (EGTA) media, the lumenal particle density increased to that of the basolateral membrane. Most likely, basolateral IMP's had invaded the lumenal membrane. Re-establishment of the junctional complex with a Ca-containing medium did not cause a reduction in lumenal IMP density for the duration of the experiment (3 hr).

More recently, Ziomek, Schulman and Edidin (1980), examined the redistribution over the cell membrane of two mouse intestinal hydrolases, alkaline phosphatase and leucine aminopeptidase, following dissociation of the epithelium into single cells by a Ca-free medium containing hyaluronidase. Histochemical and immunofluorescent staining indicated to them that both of these microvillar (i.e., apical) enzymes are found in the basolateral membrane within minutes following dissociation. The authors attributed the movement of these enzymes into the basolateral membrane to lateral diffusion, but argued that diffusion was made possible by disturbance of metabolism during dissociation, and not by disruption of the zonula occludens.

A recent fluorescence-photobleaching study on dome-forming epithelial cells in tissue culture confirms that the immobility of some epithelial membrane proteins is independent of the zonula occludens. Dragsten, Handler and Blumenthal (1982) explored the lateral mobility of membrane proteins that bind fluorescently labeled wheat germ agglutinin, a divalent lectin. After a small (<2 to 4 μm diameter) area of membrane is bleached, fluorescence apparently fails to recover even when the bleached area does not include the tight junction. Wheat germ agglutinin receptors are said to be "completely immobile," regardless of whether or not they reside on apical or basolateral sides. To the degree that Dragsten et al. (1982) can be certain that their wheat germ agglutinin receptors are predominantly integral membrane proteins rather than constituents of the basal lamina, their experiment would prove that many epithelial membrane proteins are immobile regardless of the tight junction. The authors also report that when one side of the epithelium is labeled with lectin, disruption of the tight junctions by dissociation into single cells causes lectin to spread over the entire cell. They find that after spreading, lectins are "still completely immobile."

By contrast, fluorescent lipids incorporated into epithelial cell membranes remain freely mobile. However, some lipids remain on the side of the epithelium to which they were initially supplied, as if they could not cross the tight junction. Dragsten et al. (1982) suggest that the ability of a lipid to diffuse past the tight junction depends on whether it is diffusing in the cytoplasmic or extracellular membrane leaflet.

Finally, there is evidence that even some membrane proteins may be able to pass across the zonula occludens of intact epithelia. Quaroni, Kirsh and Weiser (1979) and Hauri, Quaroni and Isselbacher (1980), using cytochemical markers, found that most (60%) of newly synthesized enzymes emerging from the Golgi apparatus appear first in the basolateral membrane and subsequently move to the microvillar or apical membrane where they apparently remain. The mechanism by which this transfer occurs is not known.

Taken together these findings suggest the following: (1) The zonula occludens can act as a barrier to lateral diffusion but is not immediately responsible for maintaining segregation of membrane proteins. Instead, membrane proteins remain segregated because they are immobile, possibly by being anchored to the cytoskeleton. (2) Disrupting the zonula occludens can lead to redistribution of membrane proteins by diffusion, but the freedom to diffuse is a transient by product of the dissociation procedure and unrelated to the disruption of the zonula occludens.

In summary, it appears that neither in epithelia nor in myelinated nerve is segregation maintained by structural barriers. There is at this point no evidence that cells use barriers to divide the cell membrane into compartments.

Conclusion

Apparently, segregation of membrane proteins is found in numerous cells. A random distribution of membrane proteins is, therefore, not a general feature in adult tissue. In most of the examples discussed, segregation is maintained by immobilizing integral membrane proteins. This may occur by linkage to other integral membrane proteins or to extra- or intracellular peripheral proteins, or combinations of the above. Thus, free mobility of membrane proteins (especially proteins involved in transport across membranes) is also not a general property of adult tissue.

Questions still remain on how segregation of membrane proteins is maintained throughout the life of an animal cell. A related question is this: how does this segregation arise in the first place? How does a cell direct the deployment of its integral membrane proteins? In many (though not all) instances, cell membrane specializations are found where neighboring cells come in close proximity and interact. This suggests that intercellular communication may be important in directing the deployment of membrane proteins. Such communication may occur in the form of synaptic transmission or by unknown "trophic factors."

Perhaps the most striking example of rapid segregation is the "capping" of lymphocytes; that is, the antibody-induced collection of cell surface antigens at one pole of the cell (Taylor, Duffus, Raff & dePetris, 1971). This process apparently involves metabolic energy as well as the interaction of actin and myosin. Whether other instances of segregation arise in similar ways remains to be seen. Useful speculation on the genesis of membrane topology can be found in a thoughtful review by Fraser and Poo (1982).

It is a pleasure to thank Dr. Bertil Hille for his critical comments on the manuscript. This work was supported by USPHS grants #AM 17803 and EY 02393.

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Received 15 March 1983; revised 28 June 1983