Membranes are Fluid Mosaics With Transient Tiles (Dynamic Domains) Defined by Curvature, Traffic and Tension

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Abstract

Cellular plasma membranes are fluid mosaics (Singer and Nicolson, 1972) that contain clearly differentiated domains or tiles, which are submicron to micron-sized areas of membrane containing a five to twenty-fold concentration of membrane proteins. Although many domains are produced by strong bonds between the membrane glycoproteins and the cytoskeleton resulting from ligand or extracellular matrix binding to the glycoprotein, there are other domains on free membranes that do not involve glycoprotein-cytoskeleton bonds. We have experimental evidence that freely diffusing membrane glycoproteins involved in cell motility are concentrated by membrane curvature (0.1-0.3 micron radius of curvature). This raises the possibility of controlling domain distributions and sizes by altering cell shape and the balance of the bilayer couple. We speculate on the basis of known membrane exchange rates and membrane structural studies that membrane traffic and membrane thickness can produce domains in flat membrane regions. Traffic and thickness domains could be controlled by altering membrane traffic and membrane tension which we have shown are linked. Our working model of the plasma membrane emphasizes the dynamic domain structures defined by membrane curvature, thickness and trafficking under the control of the intensive variables of membrane tension and bilayer couple imbalance.

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Membrane Morphology and Dynamics

The fluid nature of plasma membrane lipids allows the bilayer to follow the contours of the cytoskeleton and enables glycoproteins to diffuse laterally over many microns. Membrane bilayers lack structural integrity and generally can not hold non-spherical shapes in the presence of external forces.

Although membranes do not have significant shear elasticity, they do have a significant bending modulus and can generate significant bilayer couple forces (Sheetz and Singer, 1974). Bending stiffness and bilayer couple forces are major components in determining red cell shape although rigidification of the membrane skeleton can block shape changes. In cells with a rigid cytoskeleton, the bending stiffness and bilayer couple forces appear to play only a secondary role in overall cell shape since the forces needed to alter the cytoskeleton are greater than those needed to alter membrane shape. It is unknown whether or not bending and bilayer couple forces play a significant role in membrane trafficking or other cellular functions. Recent experimental evidence indicates that the tension within the plasma membrane is a major factor in many cellular functions, basically defining endocytosis rate and motility (Sheetz and Dai, 1996). Diffusion in plasma membranes is relatively rapid with typical diffusion coefficients in the range of 10^{-9} to 10^{-10} cm^2/sec. (Sheetz, 1993). There are, however, barriers to lateral diffusion (corrals (Sheetz, 1983)) that have been observed experimentally with single particle tracking (Sheetz et al., 1989) or with membrane drag experiments (Edidin et al., 1991). The decreased rate of glycoprotein diffusion in biological membranes compared with model bilayers is the result of viscous drag and not the corrals in the membrane except perhaps in the case of the erythrocyte (see review Sheetz (1993)). In some membranes the size of diffusional corrals can be several microns or more indicating that the attachments between the membrane and the cytoskeleton need not be numerous or strong (Kucik et al., 1990; 1991). Thus diffusion studies support a model in which the membrane is draped over the cytoskeleton raising the question of how the two structures are bonded.

Curvature-Dependent Domains

Some membrane glycoproteins are concentrated ten to twenty fold in curved regions of plasma membranes. Concentrations were observed in positively curved regions at the leading edges of lamellipodia and along filopodia in diffusion measurements of integrins (Schmidt et al., 1993) and the embryonic membrane antigen of mouse cortical neurons (Sheetz et al., 1990). Integrins are involved in motility on specific extracellular matrix molecules and their concentration at leading edges is important because those are the first regions to reach new matrix sites. The function of the embryonic membrane antigen is unknown but its localization to the leading portions of growth cones put it in a logical location to participate in pathfinding and other signaling functions. To better understand which portion of the molecule is involved in localizing the integrin ($\alpha\beta$ heterodimer) to curved regions, we observed movement of $\beta 1$ integrin with and without its cytoplasmic tail and found no difference (Schmidt et al., 1993). Concentration in positively (outward) curved regions could be a property of the external domain. It is logical to postulate that other proteins may partition to inwardly curved regions such as those in clathrin or caveolar pits. Many of the seven-transmembrane spanning receptors do partition to clathrin pits upon ligand binding and get endocytosed. Although adaptin binding to the cytoplasmic surface of the receptors may hold them in clathrin pits, they may initially partition into pits because of the negative curvature. Membranes are naturally curved when they conform to the thin regions of lamellipodia and filopodia and the energy for curving the membranes comes from the membrane-cytoskeleton interaction and tension in the membrane. Contributions from imbalances in the bilayer couple will contribute but they will be a secondary factor except in cases like the erythrocyte or where there is an extreme imbalance in the bilayer couple.

Membrane Traffic and Domains

Because many plasma membranes turn over every 30 minutes, it is possible to maintain specialized domains of the plasma membrane based upon membrane dynamics (see review by Sheetz (1993)). There are several studies of the primary exocytic and endocytic sites that show them to be localized on fibroblasts. This raises the possibility that specific glycoproteins could be added preferentially in one place and endocytosed preferentially in another. This need not represent a bulk flow of membrane, which has not been observed in fibroblasts (Kucik et al., 1990) but the flow of membrane seen in growing axons constitutes an extreme example (Dai and Sheetz, 1995). The membrane corrals would favor diffusional domain formation by increasing dramatically the time needed for membrane proteins to equilibrate over the surface by diffusion.

Membrane Thickness and Domains

It is known that bilayer thickness changes dramatically with changes in lipid state from fluid to gel. There is little reason, however, to believe that biological membrane lipids under physiological conditions will go into a gel state. However, there is an increasing interest in membrane rafts wherein proteins of similar properties but not biochemically bonded codistribute on the cell surface. One possibility is that the proteins promote a change in bilayer thickness that causes a phase separation in the plane of the membrane. Since aggregation often stimulates the coalescence of the components in the membrane, there is likely a cooperative component to the process. Although there may be mechanisms for achieving separation in the water phase, it is attractive to think of how the membrane phase could cause coalescence and membrane thickness is a property that could produce a phase separation of proteins and some lipids. Since many of the raft effects are related to cholesterol and blocked by cholesterol depletion, a mechanism based on the lipid phase is preferred.

Membrane Tension Effects on Endocytosis and Motility and Possibly Domains

A critical element in our understanding of membrane domains that has been often overlooked is tension in the plane of the membrane. For example, in the case of curvature-dependent domains, it is evident that high membrane tensions will decrease the amount of highly curved membrane. We have found that extension of lamella and filopodia is decreased dramatically at high membrane tensions resulting in a loss of those structures and consequently of curved membrane surfaces. In addition, we have found that high membrane tensions dramatically inhibit endocytosis (Dai et al., 1997) which would result in the loss of any domains produced by membrane exchange. Finally, any domain that relied upon a physical change in the bilayer such as its thickness would be sensitive to changes in membrane tension. In the case of thickness, increased tension would favor the formation of a thinner bilayer phase. Even in the case of domains formed by cytoskeletal corrals, membrane tension is a critical parameter for holding the membrane and cytoskeleton in contact and preventing bleb formation.

Membrane-Cytoskeleton Interaction and Domains

Separation of the membrane from the cytoskeleton results in bleb formation. Blebs are essentially membranous bubbles of cytoplasm without cytoskeletal support. Glycoprotein diffusion rates are increased in blebs. Disruption of actin filament organization with cytochalasin B or D stimulates blebbing which indicates that actin or actin-associated proteins such as spectrin or actin-binding protein (ABP) are important for membrane cytoskeleton adhesion. Indeed, the loss of ABP results in spontaneous bleb formation and similar behavior is seen after spectrin depletion in anti-sense experiments. The dynamic interaction between membrane and cytoskeleton contribute to many kinds of transient domains in the plasma membrane. Many different types of experiments indicate that adhesion between membrane and cytoskeleton is a result of multiple weak bonds that are dynamic. Thus, the membrane and cytoskeleton adhere nearly continuously along their interface without many high affinity bonds except at specialized adhesive contacts.

Summary

Our working model of plasma membrane structure has evolved from that of a fluid mosaic in which the membrane and the cytoskeleton are extensively bonded through multiple membrane protein-cytoskeleton linkages (which is indeed the case for the erythrocyte membrane). We now believe that a tension produced by the endocytic machinery holds the membrane on the cytoskeleton where weak, non-bonding interactions reinforce the complex. Strong membrane-cytoskeleton bonds depend upon activation of membrane proteins by ligand binding or other signals. Domains of protein concentration without cytoskeletal attachment are clearly formed by positive membrane curvature and can result from membrane dynamics with or without diffusional corrals. We speculate that negative curvature and membrane thickness can also produce membrane domains. Membrane tension is a critical intensive variable that can modulate domains either indirectly through its effects on cell motility (shape) and endocytosis or directly in the case of thickness domains.

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