Plasma Membrane Organization by the Membrane Skeleton

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Abstract

The membrane skeleton provides both corralling and binding effects on the movement of membrane proteins. I propose that these effects can play pivotal roles in the molecular organization of the plasma membrane, especially in the formation of special membrane domains.

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Biol. Skr. Dan. Vid. Selsk. 1998, **49**:137–142

Introduction

Various structures and arrays of proteins and lipids exist within and around the plasma membrane, which are essential for the proper functioning of these molecules in the plasma membrane. These supramolecular complexes include (1) multimers of receptor molecules or receptor and effector molecules, which are thought to be the first trigger for the subsequent reactions in cells after ligand binding (Grasberger et al., 1986, Metzger, 1992), (2) specialized membrane domains, such as synapses, clathrin-coated lattices and pits, caveolae, and cell-cell and cell-substrate adhesion structures, in which specific proteins and lipids are assembled to carry out specific functions (Klymkowski and Parr, 1995, Miyamoto et al., 1995), and (3) the polarized distribution of various proteins in epithelial and neuronal cells (Nelson, 1992). The constituent molecules of such supramolecular structures are recruited and assembled from the plasma membrane and the cytoplasm, and by intracellular vesicular transport. In this contribution, I am mostly concerned with the recruitment of membrane proteins within the plasma membrane.

In the recruitment, multimerization, and assembly of specific membrane proteins and lipids, one of the critical processes is the regulation of the movement of these molecules. Proteins are not free-floating in a sea of excess lipids, i.e., the cells have various means to control the mobility and specific assembly of membrane proteins into specialized domains. Of particular interest is the

involvement of membrane-skeletal elements in mediating or inhibiting movements of cell surface receptors, and their participation in the formation of specialized domains and in signal transduction in the plasma membrane (Kusumi and Sako, 1996).

Barriers to Lateral Diffusion of Membrane Receptors as Studied by Single Particle Tracking and Laser Tweezers: Fence and Tether

Movements of transferrin and alpha2-macroglobulin receptor molecules in the plasma membrane of cultured normal rat kidney (NRK) fibroblastic cells were investigated by video-enhanced optical microscopy with a nanometer-level spatial precision and a temporal resolution up to 0.2 ms by labeling the receptors with the ligand-coated nanometer-sized colloidal gold particles (Kusumi et al., 1993; Sako and Kusumi, 1994). For both receptor species, approximately 90% of the movement trajectories are of the confined diffusion type, within domains of 0.25 μm^2 (500-700 nm in diagonal length). Movement within the domains is random with a microscopic diffusion coefficient $D_{micro} \simeq 10^{-9} cm^2/s$, which is consistent with a value expected for freely diffusing proteins in the plasma membrane. The receptor molecules move from one domain to one of the adjacent domains every 25 s on average, indicating that the plasma membrane is compartmentalized for diffusion of membrane receptors and that long-range diffusion occurs as a result of successive intercompartmental hops. The macroscopic diffusion coefficients for these two receptor molecules are $\simeq 3 \times 10^{-11} cm^2/s$, which is smaller than D_{micro} by a factor of 30. The above results indicate that the macroscopic diffusion is slowed due to confinement by the boundaries, and not due to the intrinsically slow rate of diffusion. Partial destruction of the cytoskeleton and partial deletion of the cytoplasmic domains of many membrane receptors strongly influenced their diffusion properties, indicating that the boundaries between compartments are made of the membrane-associated part of the cytoskeleton or the membrane skeleton (membrane-skeleton fence model).

The mechanical properties of intercompartmental boundaries were then studied by tagging transferrin receptor (TR) with either 210 nm latex or 40 nm colloidal gold particles, and by dragging the particle-TR complexes laterally along the plasma membrane using laser tweezers (Sako and Kusumi, 1995). Approximately 90% of the TR-particle complexes, which showed confined-type diffusion with D_{micro} of $\simeq 10^{-9} cm^2/s$, could be dragged past the intercompartmental boundaries in their path by laser tweezers at a trapping force of 0.35 - 0.8 pN. At the dragging forces between 0.05 and 0.1 pN, particle-TR complexes tended to escape from the laser trap at the boundaries, and such escape occurred in both the forward and backward directions of dragging. The boundaries are elastic with an effective elastic constant of 1-10 $pN/\mu m$. These results are consistent with the proposal that the compartment boundaries consist of membrane skeleton. Approximately 10% of TR exhibited slower diffusion $D_{micro} \simeq 10^{-10} - 10^{-11} cm^2/s$ and binding to elastic structures.

Regulation of the Movements of Erythrocyte Band 3 As Studied by Single Particle Tracking and Laser Tweezers

We have proposed a "membrane-skeleton fence model", in which close apposition of the membrane skeleton meshwork to the membrane gives effective barriers for free diffusion of membrane proteins due to steric hindrance. This model was examined by single particle tracking with a high speed camera and by laser tweezers.

(1) Erythrocyte band 3 was labelled by paucivalent colloidal gold. The mobile fraction of band 3 was 65% at 37°C. These molecules undergo free diffusion $D_{\text{micro}} \simeq 5.3 \times 10^{-9} \text{cm}^2/\text{s}$ within domains of $\approx$ 110-nm in diameter, and hop to adjacent domains every 350 ms on average. D_{macro} was one-sixtieth of D_{micro}.

(2) The cytoplasmic domain of band 3 was removed by brief trypsin treatment, which did not cleave spectrin and actin. The domain size and D_{micro} was the same after cleavage, but only the hop rate increased by a factor of 6 to once every 60 ms on average.

(3) When the membrane skeleton was dragged laterally by optical tweezers via attached latex bead (1 micron-diameter), mobile band 3 was also moved along with the membrane skeleton. This result indicates collision of band 3 with the membrane skeleton.

These results support the membrane skeleton fence model.

Regulation of Band 3 Diffusion by Dissociation-Association Equilibrium of the Erythrocyte Membrane Skeleton

The mechanism of intercompartmental hop of band 3 was investigated using optical tweezers.

(1) Band 3 was dragged along the membrane by optical tweezers at several different scan rates. At velocities lower than 1.6 μm/s, band 3 could be moved freely. But at velocities higher than 1.6 μm/s, band 3 often escaped from the trap. These results suggest that, when dragged at a velocity lower than 1.6 μm/s, band 3 tends to pass a fence before band 3 collides with the next fence, and that the fence undergoes conformational change every 70 ms on average (= 110 nm / 1.6 μm/s) that allow the passage of band 3.

(2) The conformational change may be either dissociation of spectrin from tetramers to dimers or conformational fluctuation of the tetramers. When the membrane skeletal network was dragged and elongated by optical tweezers, the hop rate increased for the elongated fence. Since the fluctuation of spectrin should be smaller when spectrin is elongated, the intercompartmental hop of band 3 is likely to be caused by dissociation of spectrin tetramers to dimers.

We propose that the passage of band 3 over the spectrin fence is facilitated by dissociation of spectrin tetramers to dimers, which takes place on the average of once every 70 ms.

Membrane-Skeleton Fence Model

Since variations in the particle size (40 and 210 nm; the particles are on the extracellular surface of the plasma membrane) hardly affect the diffusion rate and behavior in the dragging experiments, and since treatment with either cytochalasin *D* or vinblastin affects the movements of TR, the boundaries are likely to be present in the cytoplasmic domain. The rebound motion of the particle-TR complexes when they escape from the laser tweezers at the compartment boundaries suggests that the boundaries are elastic structures. These results are consistent with the proposal that the compartment boundaries consist of a membrane-associated portion of the cytoskeleton (membrane-skeleton fence model).

In this model, the membrane skeleton provides a barrier to free diffusion of membrane proteins due to steric hindrance (the space between the membrane and the cytoskeleton is too small to allow the cytoplasmic portion of the membrane protein to pass), thus compartmentalizing the membrane into many small domains of 0.1 - 1 μm^2. The membrane proteins can escape from one domain and move to adjacent compartments due to the dynamic properties of the membrane skeleton: the distance between the membrane and the skeleton may fluctuate over time (or the membrane skeleton may dissociate from the membrane), or the membrane-skeleton network may form and break continuously due to dissociation-association equilibrium, thus giving the membrane proteins an opportunity to pass through the mesh barrier. Furthermore, the membrane protein molecules that have sufficient kinetic energy will be able to cross the boundaries.

Confined lateral diffusion and intercompartmental hop diffusion of membrane proteins have been observed in a variety of membrane proteins in all cells studied thus far. We propose that compartmentalization of the plasma membrane by a membrane-skeleton/cytoskeleton meshwork (membrane-skeleton fence structure) is a basic feature of the plasma membrane. For individual protein species, more specific mechanisms such as direct binding to the cytoskeleton may be at work. However, what should be emphasized here is that the fence effect of the membrane skeleton is superimposed on the specific effect for individual protein species. In the case of E-cadherin, some molecules that are bound to the flexible cytoskeleton (possibly thin actin filaments) "feel" the presence of the membrane skeleton fence as they move about with the attached cytoskeleton (Sako and Kusumi, unpublished observation).

Binding and Transport of Membrane Proteins by the Membrane Skeleton

Binding of membrane proteins to the membrane skeleton has been found for almost all proteins investigated so far, including the receptors for transferrin, EGF, and alpha2-macroglobulin, E- and T-cadherins, and band 3 anion channel in erythrocytes. (The strength of the interaction between the cytoskeleton and the membrane bilayer has been estimated to be 2 - 8 pN.) These bound proteins undergo various types of motion. Some show no motion, while some show oscillatory movements without real translation. Some proteins show long-range translational diffusion while they are apparently bound to the membrane skeleton (which is known because these particles cannot be dragged more than 100 nm by laser tweezers). Some show directed transport-type movements probably due to the active movement of the cytoskeleton to which they are bound.

The mechanism by which the cells control these processes and exert the fence effect of the membrane skeleton has yet to be elucidated. The amount of E-cadherin bound to the membrane-

skeleton decreases after a calcium switch. The size of the compartment as "felt" by the Na, K-ATPase in the dorsal/apical membrane decreases by a factor of 2 after the calcium switch in MDCK cells. Specific binding to a particular membrane skeleton/cytoskeleton may be controlled by phosphorylation. It is likely that cells are using the fence effect and active transport by the membrane-skeleton/cytoskeleton to assemble specific membrane proteins into specialized domains. However, exactly how cells do this is not known and is one of the most important issues in membrane biology.

Control Mechanisms for the Formation of Supramolecular Arrays and Assemblies in and around the Plasma Membrane

We envisage three basic processes for the assembly of membrane proteins through movements in the plasma membrane. These are basic concepts and are not mutually exclusive. (1) Diffusion of the membrane protein and entrapment at specific sites in the membrane can occur, possibly due to preassembly of cytoplasmic proteins on the cytoplasmic surface of the membrane. Cooperative assembly of intramembrane proteins and peripheral proteins is a possibility. (2) Cells take advantage of thermal diffusion to drive the movements of membrane proteins, but regulate the direction of the movements by varying the structure of the membrane skeleton using free energy released by decomposition of ATP. In this working hypothesis, we postulate that the free energy generated by ATP decomposition is not used to drive the movements but to regulate the movements. The basic idea for this hypothesis is that rather than simple self-assembly of molecules, cells actively regulate thermal movements to construct supramolecular complexes. (3) Gross, active movements of the membrane-skeleton network may occur to move the membrane proteins trapped in the compartments (like many fish in a fishnet) or those bound to the skeletal network. For example, we envisage that oligomers and aggregates are bound to the membrane skeleton and are carried by the skeleton as a single cargo. The key idea is that cells would not move membrane proteins one by one because it is energetically and temporally too inefficient.

We believe that the cytoskeleton/membrane-skeleton works as an organizer of molecules in the plasma membrane. In addition to regulating the movements of membrane proteins, the membrane-skeleton/cytoskeleton may regulate other undercoat structures of the plasma membrane such as caveolae and clathrin-coated structures.

References

Gadella, T.W.J., and T.M. Jovin. 1995. Oligomerization of epidermal growth factor receptors on A431 cells studied by time-resolved fluorescence imaging microscopy. A stereochemical model for tyrosine kinase receptor activation. *J. Cell. Biol.* 129:1543-1558.

Grasberger, B., A.P. Minton, C. DeLisi, and H. Metzger. 1986. Interaction between proteins localized in membranes. *Proc. Natl. Acad. Sci. USA* 83:6258-6262.

Klymkowski, M.W., and B. Parr. 1995. The body language of cells: the intimate connection between cell adhesion and behavior. *Cell* 83:5-8.

Kusumi, A., Y. Sako, and M. Yamamoto. 1993. Confined lateral diffusion of membrane receptors as studied by single particle tracking (nanovid microscopy). Effects of calcium-induced differentiation in cultured epithelial cells. *Biophys. J.* 65:2021-2040.

Kusumi, A., and Y. Sako. 1996. Cell surface organization by the membrane skeleton. *Curr. Opinion Cell Biol.* 8:566-574.

Metzger, H. 1992. Transmembrane signaling: the joy of aggregation. *J. Immunol.* 149:1477-1487.

Miyamoto, S., S.K. Akiyama, and K.M. Yamada. 1995. Synergistic roles for receptor occupancy and aggregation

in integrin transmembrane function. *Science* 267:883-885.

Nelson, W.J. 1992. Regulation of surface polarity from bacteria to mammals. *Science* 258:948-955.

Sako, Y., and A. Kusumi. 1994. Compartmentalized structure of the plasma membrane for receptor movements as revealed by a nanometer-level motion analysis. *J. Cell Biol.* 125:1251-1264.

Sako, Y., and A. Kusumi. 1995. Barriers for lateral diffusion of transferrin receptor in the plasma membrane as characterized by receptor dragging by laser tweezers: fence versus tether. *J. Cell Biol.* 129:1559-1574.