Chernomordik, L. V., M. M. Kozlov, G. B. Melikyan, I. G. Abidor, V. S. Markin, and Y. A. Chizmadzhev. 1985. The shape of lipid molecules and monolayer fusion. *Biochim. Biophys. Acta.* 812:643–655.

Chernomordik, L. V., G. B. Melikyan, and Y. A. Chizmadzhev. 1987. Biomembrane fusion: a new concept derived from model studies using two interacting planar lipid bilayers. *Biochim. Biophys. Acta.* 906:309–352.

Helfrich, W. 1973. Elastic properties of lipid bilayers: theory and possible experiments. *Z. Naturforch.* 28c:693–703.

Kozlov, M. M., S. L. Leikin, L. V. Chernomordik, V. S. Markin, and Y. A. Chizmadzhev. 1989. Stalk mechanism of membrane fusion. Intermixing of aqueous contents. *Eur. Biophys. J.* 129:411–425.

Leikin, S. L., M. M. Kozlov, L. V. Chernomordik, V. S. Markin, and Y. A. Chizmadzhev. 1987. Membrane fusion: overcoming of the hydration barrier and local restructuring. *J. Theoret. Biol.* 129:411–425.

Markin, V. S., M. M. Kozlov, and V. L. Borovjagin. 1984. On the theory of membrane fusion. The stalk mechanism. *Gen. Physiol. Biophys.* 5:361–377.

Nanavati, C., and J. M. Fernandez. 1993. The secretory granule matrix: a fast acting smart polymer. *Science (Wash. DC).* 259:963–965.

Nanavati, C., V. S. Markin, A. F. Oberhauser, and J. M. Fernandez. 1992. The exocytotic fusion pore modeled as a lipidic pore. *Biophys. J.* 63: 1118–1132

Rand, R. P., and V. A. Parsegian. 1986. Mimicry and mechanism in phospholipid models of membrane fusion. *Annu. Rev. Physiol.* 48: 201–212.

Verkleij, A. J., C. Mombers, W. J. Geritsen, J. Leunissen-Bijvelt, and P. Cullis. 1979a. Fusion of phospholipid vesicles in association with the appearance of lipidic particles as visualized by freeze fracturing. *Biochim. Biophys. Acta.* 555: 358–361.

Verkleij, A. J., C. Mombers, J. Leunissen-Bijvelt, and P. H. Verbergaert. 1979b. Lipidic intramembraneous particles. *Nature (Lond.).* 279: 162–163.

A Closer Look at How Membrane Proteins Move

Akira Ishihara* and Ken Jacobson*‡

*Department of Cell Biology and Anatomy and ‡Lineberger Cancer Research Center University, North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599 USA

The lateral diffusion of many plasma membrane proteins is impeded and a significant percentage of those proteins appear virtually immobile. This restricted mobility is commonly believed to result directly or indirectly from interactions of proteins with the underlying cytoskeleton (Zhang et al., 1993). Ultrastructure studies have shown that in red blood cells as well as other cells a regular spectrin lattice is attached to the membrane (Pumplin and Bloch, 1993). In spectrin-deficient red blood cells, a large increase in the diffusion rates of membrane proteins was observed (Sheetz et al., 1980). These findings, together with other support, led to the view that proteins are corralled within membrane domains by interactions of their cytoplasmic moiety with cytoskeletal lattices (Sheetz, 1983; Tsuji and Ohnishi, 1986; Tsuji et al., 1988; Saxton, 1990). However this model has not been confirmed by direct experiment.

Single Particle Tracking (SPT) is a recently developed technique, in which membrane proteins or lipids are specifically labeled with antibody-coated submicron colloidal gold or fluorescent latex particles and the trajectories of the individual labeled molecules are followed by digital imaging microscopy (Zhang et al., 1993; Cherry, 1992; Ghosh and Webb, 1990). By direct observation of individual protein motions, the method should provide detailed information on submicroscopic membrane structures, although careful data analysis is required in order to distinguish trajectories of nonrandom motions from those of random motions (Saxton, 1993). Kusumi et al (1993) applied this new technique to study the mobility of E-cadherin, a Ca^{2+}-dependent cell adhesion molecule, in cultured epidermal cells. Trajectories were carefully analyzed with the help of computer simulations. The results give a strong evidence for the notion that some membrane proteins are confined within certain regions.

The results of Kusumi et al. (1993), as well as earlier work on the low-density lipoprotein receptor (Ghosh and Webb, 1990; Anderson et al., 1992), indicate that the SPT method can, indeed, provide more detailed information about membrane protein mobility. The more conventional method, fluorescence recovery after photobleaching, typically divides the labeled protein population into mobile and immobile fractions. For E-cadherin, 25% was immobile and the remaining 75% was characterized by a diffusion coefficient of 2.6×10^{-11} cm^2/s. On the other hand, the SPT method revealed that four different modes of the membrane protein mobility were operant. More than half the proteins tracked (64%) showed diffusion confined to regions on the order of 500 nm or less. 6% of the population was virtually stationary, while 2% showed directed motion. The remaining 28% of the population exhibited random diffusion.

To further characterize random diffusion, "microscopic diffusion coefficients," which reflect the mobility on the tens of nanometer scale, were calculated from individual protein trajectories. These values were distributed over a surprisingly large range of 4.6×10^{-12} to 1×10^{-9} cm^2/s, but it is not yet known how much of this variation is real and how much is statistical. Directed motion has been observed previously and is presumably due to the interaction with the dynamic cytoskeleton system (De Brabander et al., 1991; Sheetz et al., 1989). However, what is most striking is that many of the tracked proteins appear to be trapped within small regions. The size of these regions was variable, ranging between 0.3 and 0.6 μm in diameter. This domain size is slightly smaller than that previously estimated by Edidin et al (1991) (0.6 μm) and larger than the estimated size of the spectrin mesh in red blood cells (approximately 0.1 μm).

Do all membrane proteins experience such confined diffusion in the plasma membrane? Kusumi et al. (1993) found that epidermal growth factor and transferrin receptors do, which is plausible because these proteins, as well as E-cadherin, possess relatively large cytoplasmic domains. Indeed, truncation of the cytoplasmic domains of E-cadherin results in much less confined diffusion (Sako et al., 1992). Whether proteins with smaller cytoplasmic domains experience such direct cytoskeletal interactions or more indirect effects is not yet known (Zhang et al., 1993). Laser trap evidence suggests that glycosylphosphatidylinositol-anchored

Received for publication 13 September 1993.

0006-3495/93/11/1754/08 $2.00

proteins are considerably less likely to be trapped in smaller membrane domains (Edidin et al., 1991) and SPT of lipid analogs shows no evidence of confinement to plasma membrane domains (Lee et al., 1993).

There is a significant discrepancy between the SPT and photobleaching results: membrane proteins in the stationary mode (6%) cannot account for the larger immobile fraction (25%) measured by photobleaching. Kusumi et al (1993) argue that a portion of the confined diffusion mode could account for the rest of the immobile fraction. This is because the area bleached by the laser (0.63 μm^2) is significant larger than the putative membrane domains, so that some bleached regions won't have a contiguous reservoir to sustain fluorescence recovery. However, if all the proteins in the confined diffusion mode are assumed to contribute to the immobile fraction, it becomes far greater than the value measured by photobleaching. This problem can be resolved if about half of the proteins in the confined diffusion mode could escape to adjacent domains. The authors propose a model in which the fences that bound a membrane domain are dynamic, having unspecified "gates" that open temporarily. In this way a single protein, which is confined much of the time, may move long distances in the membrane plane. Indeed, unpublished studies indicate that the labeled E-cadherin shows intercompartmental movements (Kusumi et al., personal communication). Whether a "gate" allows "escape" of a given protein to an adjacent domain will depend on the size of its cytoplasmic moiety, so that the effective domain size may depend on the protein. This would explain why domain sizes differ for various proteins.

Those who are fascinated by the complex dynamics of plasma membranes will eagerly wait additional detailed SPT studies of other membrane proteins, for they will most certainly enhance our understanding of this organelle which is so pivotal in modern cell biology.

REFERENCES

Anderson, C. A., G. N. Georgion, I. E. G. Morrison, G. V. W. Stevenson, and R. Cherry. 1992. Tracking of cell surface receptors by fluorescence digital imaging microscopy using charge-coupled device camera. Low density lipoprotein and influenza virus receptor mobility at 4 C. *J. Cell Sci.* 101:415–425.

Cherry, R. J. 1992. Keeping track of cell surface receptors. *Trends Cell Biol.* 2:242–244.

De Brabander, M., R. Nuydens, A. Ishihara, B. Holifield, K. Jacobson, and H. Greets. 1991. Lateral diffusion and retrograde movements of individual cell surface components on single motile cells observed with nanovid microscopy. *J. Cell Biol.* 112:111–124.

Edidin, M., S. C. Kuo, and M. P. Sheetz. 1991. Lateral movements of membrane glycoproteins restricted by dynamic cytoplasmic barriers. *Science (Wash. DC).* 254:1379–1382.

Ghosh, R. N., and W. W. Webb. 1990. Evidence for intra-membrane constraints to cell surface LDL receptor motion. *Biophys. J.* 57:286*a*. (Abstr.)

Lee, G. M., F. Zhang, A. Ishihara, C. L. McNeil, and K. A. Jacobson 1993. Unconfined lateral diffusion and an estimate of pericellular matrix viscosity revealed by measuring the mobility of gold-tagged lipids. *J. Cell Biol.* 120:25–35.

Kusumi, A., Y. Sako, and M. Yamamoto. 1993. Confined lateral diffusion of membrane receptors as studied by single particle tracking (nanovid microscopy). *Biophys. J.* 65: 2021–2040.

Pumplin, D. W., and R. J. Bloch. 1993. The membrane skeleton. *Trends Cell Biol.* 3:113–117.

Sako, Y., A. Nagafuchi, M. Takeichi, and A. Kusumi. 1992. Intracellular regulation of the movements of E-cadherin on the cell surface. *Mol. Biol. Cell* 3:219*a*. (Abstr.)

Saxton, M. J. 1990. The membrane skeleton of erythrocytes:models of its effect on lateral diffusion. *Int. J. Biochem.* 22:801–809.

Saxton, M. J. 1993. Lateral diffusion in an archipelago: single particle diffusion. *Biophys. J.* 64:1766–1780.

Sheetz, M. P., M. Schindler, and D. E. Koppel. 1980. Lateral mobility of integral membrane proteins is increased in spherocytic erythrocytes. *Nature (Lond.).* 285:510–512.

Sheetz, M. P. 1983. Membrane skeletal dynamics: role in modulation of red cell deformability, mobility of transmembrane proteins, and shape. *Semin. Hemat.* 20:175–188.

Sheetz, M. P., S. Turney, H. Qian, and E. L. Elson. 1989. Nanometre-level analysis demonstrations that lipid flow does not drive membrane glycoprotein movements *Nature (Lond.).* 340:284–288.

Tsuji, A., and S. Ohnishi. 1986. Restriction of the lateral motion of band 3 in the erythrocyte membrane by the cytoskeletal network: dependence on spectrin association state. *Biochemistry.* 25:6133–6139.

Tsuji, A., K. Kawasaki, S. Ohnishi, H. Merkle, and A. Kusumi. 1988. Regulation of band 3 mobilities in erythrocyte ghost membranes by protein association and cytoskeletal meshwork. *Biochemistry.* 27:7447–7452.

Zhang, F., G. M. Lee, and K. Jacobson. 1993. Protein lateral mobility as a reflection of membrane microstructure. *BioEssays.* 15:579–588.

Distribution of Voltage Sensors in Mammalian Outer Hair Cells

Daniel L. Alkon,* René Etcheberrigaray,* and Eduardo Rojas‡

* Laboratory of Adaptive Systems, National Institute of Neurological Disorders and Stroke, and ‡ Laboratory of Cell Biology and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20852, USA

It is now widely accepted that outer hair cells (OHC) play a fundamental role in normal cochlear transduction (Brownell et al., 1985; Ashmore, 1987; Kalinec et al., 1992). Understanding how the behavior of the OHC determine the critical frequency selectivity of the mammalian cochlea, however, has been a more recent development to which the article in this issue by Huang and Santos-Sacchi (p. 2228) makes an outstanding contribution.

In mammals, the cochlea separates sound frequencies by controlling the motion of the basilar membrane. It has become increasingly clear that a metabolically labile process involving the OHC greatly contributes to this motion control of the basilar membrane (Holley and Ashmore, 1988; Iwasa and Kachar, 1988; Santos-Sacchi, 1991). The OHC exhibit electrically induced elongation and contraction movements that enhance the frequency selectivity and sensitivity of the basilar membrane movement wave. Several lines of evidence have shown that the OHC movements depend on or are sensitive to changes in membrane potential (Iwasa and Kachar, 1988). This discovery suggested the presence of a voltage-acting molecule within the plasma membrane of the OHC. The presence of a voltage-dependent nonlinear charge movement as manifest by a voltage-dependent capacitance has provided additional evidence for the existence of such a molecule (Santos-Sacchi, 1991).

Electrophysiological and microscopy studies (Kalinec et al., 1992) have shown that OHC elongation in response to hyperpolarization and depolariza-

Received for publication

0006-3495/93/11/1755/08 $2.00