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 Indeed, unpublished studies indicate that the labeled E-cadherin shows intercompartmental movements (Kusumi et al., personal communication).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-

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tion-induced shortening, occur at the lateral plasma membrane. These high frequency movements would involve a voltage-sensitive force generator located in the lateral membrane and a specialized cytoskeleton beneath that maintains the cylindrical shape of the cell, ensuring that forces produced in the membrane cause changes in cell length. It has also been determined that the mechanical responses of the OHC occur only in the central region of the cell (Dallos et al., 1991). The present study in this issue of *Biophysical Journal* mainly focuses on the voltage sensor and its localization.

OHC were acutely isolated by a combination of mechanical and enzymatic treatment. For recordings, cells were maintained in saline solution with ion current blockers (tetraethylammonium, tetrodotoxin, and $CoCl_2$) to avoid interference with capacitive current measurements. A protocol involving the use of a partitioning microchamber and a double-voltage clamp was used to electrically amputate and measure cell capacitance in different regions of the OHC.

By analogy with the two classical models of signal transduction which involve confined displacements of charged regions of a protein molecule (the voltage sensor), namely Na^+-channel gating (Armstrong and Bezanilla, 1974; Keynes and Rojas, 1974) and excitation-contraction coupling (Schneider and Chandler, 1973), the authors concluded that in OHCs the physical domain of the nonlinear charge movement corresponds to that of the mechanical effector. This physical domain apparently occurs in the plasmalemma of the cell's central portion and not the apical and basal portions.

Mechanosensory frequency filtering, thus provides a new example of signal transduction involving confined displacements of charged regions of protein molecules. Multiple applications for this single type of molecular transduction strategy provide another remarkable instance of nature's economy of function.

REFERENCES

1. Armstrong, C. M., and F. Bezanilla. 1974. Charge movement associated with the opening and closing of the activation gates of Na channels. *J. Gen. Physiol.* 63:533–552.
2. Ashmore, J. F. 1987. A fast motile response in ginea-pig outer hair cells: the cellular basis of the cochlear amplifier. *J. Phisiol. (Lond.).* 388:323–347.
3. Brownell, W. E., C. R. Bader, D. Bertrand, and Y. de Ribaupierre. 1986. Evoked mechanical responses of isolated cochlear outer hair cells. *Science (Wash. DC).* 227:194–196.
4. Holley, M. C., and J. F. Ashmore. 1988. On the mechanism of a high-frequency force generator in outer hair cells isolated from the guinea pig cochlea. *Proc. R. Soc. Lond. B.* 232:413–429.
5. Huang, G., and J. Santos-Sacchi. 1993. Mapping the distribution of the outer hair cell motility voltage-sensor by electrical amputation. *Biophys. J.* 65:2228–2236.
6. Iwasa, K. H., and B. Kachar. 1989. Fast in vitro movement of outer hair cells in an external electric field: effect of digitonin, a membrane permeabilizing agent. *Hearing Res.* 40:247–254.
7. Kalinec, F., M. C. Holley, K. H. Iwasa, D. L. Limb, and B. Kachar. 1992. A membrane-based force generation mechanism in auditory sensory cells. *Proc. Natl. Acad. Sci. USA.* 89:8671–8675.
8. Keynes, R. D., and E. Rojas. 1974. Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axon. *J. Physiol. (Lond.).* 239: 393–434.
9. Schneider, M. F., and W. K. Chandler. 1973. Voltage-dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. *Nature (Lond.).* 242: 244–246.