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 $(0.63 \,\mu m^2)$ 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 Indeed, unpublished studies indicate that the labeled E-cadherin shows intercompartmental movements (Kusumi al., personal communication). et 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.

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Distribution of Voltage Sensors in Mammalian Outer Hair Cells

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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 voltagedependent 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 CoCl₂) 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 in-

volve 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.

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