New and Notable

Everything You Always Wanted to Know about Sachs' Seals

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It is impossible to overstate the importance of PCR to biological science. I refer, of course, to patch-clamp recording. This electrophysiological technique, developed in the late 1970s for the rather narrow purpose of directly observing single ion channel currents, has laid down deep tracks throughout biology by opening up a multitudinous sea of cell types—systems beyond the squid axon and the neuromuscular junction—to precise measurement of membrane electrical properties. The consequences have been immense for fields as diverse as cell signaling, analysis of membrane protein mechanism, physiology of hormone secretion, and the molecular underpinnings of long-lasting neuronal information storage.

Patch recording requires a patch: a tiny area, ~1 μm^2, of cell membrane that becomes electrically sealed off from the extracellular solution when kissed by a glass micropipette and gently wooed into an intimate embrace with the electrode's glass surface—the tighter the seal, the better the recording. In 1980, a wonderful miracle occurred when Hamill et al. (1) discovered conditions under which a seal of very high resistance—a ''gigaseal''—could be made to form between the electrode wall and the cell membrane. Like all miracles, this one has remained mysterious over the years. What kinds of forces dictate the bond of a lipid membrane to glass? How is it even possible for a biological membrane, all fuzzed up with protruding proteins, to avoid electrically catastrophic leaks in such a bond? What kind of new environments do proteins experience when they suddenly find themselves sucked up into a membrane patch? Are these patches as physically homogeneous as in the familiar cartoons depicting them as omega-shaped domes blebbing into the pipette tip?

For more than a decade, Fred Sachs, a pioneer in the field of mechanosensitive ion channels, has been combining electrical and microscopic methods to tease out fine details of gigaseal formation, for quantifying membrane tension (2), and improving the reliability of seal formation (3). In this issue, he and his colleagues (4) push these efforts forward with a provocative potpourri of patch-personality presentations. Electrophysiologists know well how viciously variable in behavior patches can be with day of the week or phase of the moon; most of us just keep plugging along until the ''good day'' or the ''good plate of cells'' raises our spirits (and our experimental productivity). This study examines patches up close with high-resolution optical microscopy and fluorescently labeled proteins to understand some of this variability. The authors, expert gigaseal psychoanalysts, reveal an amazing variety of patch behavior: seals that form smoothly; seals that form in heterogeneous ruffles and either stay that way, useless, or smooth up after being ''exercised'' by pressure pulses in a kind of patch-pacifying, two-dimensional yoga; seals that exclude specific proteins; seals that gather up other proteins; seals that creep; seals that balloon up; seals that flatten down; and, of course, seals that just break and end the experiment.

What is a seal, actually? A quick calculation shows that a 50-Å slab of extracellular solution between the membrane and the glass wall—the width of a typical protrusion of a membrane protein—would be much leakier than is observed in good seals. So what is in there, and what happens to the membrane proteins? The authors posit in their ''fried egg'' model that, depending on cell characteristics, seals are plugged up with a viscous, denatured glycoprotein grout that greatly retards ionic flow. Moreover, they argue that the forces holding the seal are mainly van der Waals in character, balanced by electrostatic repulsion between the negatively charged surfaces of glass and membrane. The electroosmotic behavior shown here provides support for this picture and helps to explain the well known, annoying behavior of patches at high voltages, nonphysiological ionic strengths, and pathological pH values.

The results also provide cautionary images for any membrane protein that envisages a patch as faithfully representing the whole-cell membrane in which it normally dwells. In the patch, that protein is subject to all sorts of novel and potentially unpleasant experiences: disruption of cytoskeletal connections, repartitioned lipid phases, unwonted resting tension approaching membrane rupture, protein crowding, and—the biophysicist's bane—deep invaginations with high series resistance. The basic message is: patches are eminently useful and informative devices—but don't get complacent.

And after you have read the study, see the movies! They are embedded in the Supporting Material and will provide hours of family-appropriate fun.

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