Molecular Clues to Mechanosensitivity

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Mechanosensitivity is a likely feature of all living cells including both prokaryotes and eukaryotes. This ubiquity may relate to an early requirement of primitive cells to sense changes in membrane tension in order to volume regulate during osmotic stresses. Although single mechanosensitive (MS) membrane ion channels have been described in many patch-clamp studies, a major advance in the field would come with the identification, cloning, and expression of the MS channel genes(s). In this issue, Sukharev et al. (1) report their initial steps in identifying MS channel proteins in *Escherichia coli* and functionally reconstituting them in liposomes. This represents the first report of actual protein involvement in MS channel activity and will presumably lead to cloning and sequencing of the appropriate prokaryotic genes. Recently, other laboratories using quite different strategies, namely isolation of genetic mutants (2) and expression cloning (3, 4), have made significant steps toward this goal for putative MS channels and a volume-sensitive channel in eukaryotic cells.

In the past, one hindrance in the isolation and identification of MS channel proteins has been the absence of high affinity/specificity ligands. Such ligands proved critical in the labeling and purification of other membrane channel proteins. In particular, tetrodotoxin for the voltage-gated Na^+ channel, α-bungarotoxin for the acetylcholine receptor channel, and amiloride for the epithelial Na^+ channel. In spite of the lack of an appropriate ligand, Sukharev et al. (1) have reconstituted partially purified MS channel proteins in liposomes and assayed for single MS channel activity with patch-clamp recording. Using detergent-solubilized membrane proteins extracted from a mutant strain of *E. coli* (deficient in the normally predominant membrane porin proteins) they were able to separate and reconstitute two functional classes of MS channels located in two broad protein fractions (60–80 and 200–400 kDa). The reconstituted MS channels displayed similar but not identical properties to those seen in native *E. coli* membranes.

The liposome reconstitution experiments also have implications for the MS gating mechanism in *E. coli*. Two different mechanisms of MS channel gating have been proposed (see Ref. 5). In one, gating tension is exerted through membrane-cytoskeleton interactions, while in the other the tension is exerted solely within the membrane bilayer. The two mechanisms need not be mutually exclusive, however, the results of Sukharev et al. showing MS channel activity in presumably cytoskeleton-free liposomes indicate that tension developed in the bilayer is sufficient to gate the MS channel in the bacterial cell. Although this gating mechanism may apply to prokaryotes there are various lines of evidence (5, 6) that argue for involvement of the cytoskeleton in eukaryote MS channel gating, which may also explain the differences in the sensitivity and dynamic properties of MS channels in eukaryote versus prokaryote cells. For example, pressure-clamp/patch-clamp studies (7) of *Xenopus* oocytes indicate that the high sensitivity and rapid adaptive behavior of MS channels can be progressively removed by decoupling the membrane from the underlying cytoskeleton during patch recording (6). Interestingly, in the case of *E. coli*, mechanosensitivity seems to increase in the cytoskeleton-free liposome versus the native membrane (1). Clearly, a number of loose ends exist in terms of understanding the mechanism(s) of mechanogating in any cell type. For example, a striking but as yet unexplained feature of MS channel gating in both prokaryotes (1) and eukaryotes (6) is their unusual voltage dependencies.

As mentioned above, other studies have provided clues to the molecular nature of MS channels in eukaryotes. For example, prompted by the observation that amiloride blocks MS channels in hair cells, Hackney et al. (8) used antibodies to the amiloride-sensitive epithelial Na^+ channel to immunolocalize putative MS channels on the hair cell cilia and identify a 62–64-kDa band in immunoblots of cochlear tissue (8). This antibody cross-reactivity indicated at least some structural relationship between the epithelial Na^+ channel and the putative hair cell MS channel. This idea was recently reinforced by the finding that the cloned epithelial Na^+ channel gene (3) displays extensive homology with specific *mec* genes in *Caenorhabditis elegans*, hypothesized to code for proteins involved in mechanotransduction (2, 9). The gene sequences predict protein products of 79 kDa and around 85 kDa for the epithelial Na^+ channel (3) and mec proteins (9), respectively. Although these most recent studies are exciting it has yet to be shown that the gene products are themselves MS channels.

A central issue regarding MS channels is their functional significance in nonsensory cells. As mentioned above, MS channels have been implicated in volume regulation during osmotic stress. A wide variety of different volume-sensitive membrane conductances have been identified including those which are cation-, K^+-, and Cl^--selective. Although some volume-sensitive channels have been shown to be also activated by direct membrane stretch, there also appear to be volume-sensitive channels that are not stretch-sensitive (see references in Ref. 4). Recently, the gene coding for a volume-sensitive Cl^- channel has been cloned and expressed in *Xenopus* oocytes (4). Structure-function analysis of different domains of the molecule indicate that a cytoplasmic tethered "ball" may normally inactivate or block the channel, but it is removed by hyperpolarization or hypotonicity (cell swelling) (4). Therefore the gating of specific volume-sensitive channels may be mediated by electrical and/or biochemical mechanisms as well as by direct mechanical means.

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