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Topical Review

Mechanosensitive Ion Channels

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Introduction

Cells are seldom static. They grow, migrate, contract, change volume and shape, reorganize internally, divide, and are subject to all manner of compression, shearing and stretch. Evidence is growing that most cells have ion channels potentially capable of monitoring and regulating active and passive variations in cellular mechanics. The ion channels in question are mechanosensitive (MS), that is, their open probability (P_{op}) depends on stress at the membrane (*see* Fig. 1 for examples). Channels of this ilk were postulated decades ago as a way to account for mechanoelectrical transduction in muscle spindles, crustacean stretch-receptors, Pacinian corpuscles and other specialized mechanoreceptors [*see* references in 17, 41–45]. What was not anticipated was the picture now emerging, with MS channels present as membrane components in ordinary pedestrian cells, rather than being confined to the exotic mechanoreceptor specialists.

As documented in other recent reviews [27, 44, 49], MS channels of various ionic selectivities have been found in spheroplasts prepared from bacteria and fungi, in protoplasts prepared from plants, and in a multitude of animal cells of vertebrate and invertebrate origin. Presently, the classification *MS*, which encompasses stretch-activated (SA) and stretch-inactivated (SI) [38] and possibly other channels (Table 1), is merely phenomenological; procaryotic and eucaryotic MS channels, in particular, may be quite unrelated. Commonality of gating mode need not signify a common underlying mechanism.

The fundamental biophysical question about MS channels—how does mechanical disturbance of the membrane alter P_{op}?—remains unanswered. Likewise, major physiological questions have yet to be addressed; there has been no unequivocal demonstration at the single channel level that MS channel *X* plays functional role *Y* in any cell. There are, however, a wealth of observations, with no shortage of interesting biophysical and physiological speculations [44]. Sachs, who, with his co-workers [17], first characterized SA channels in chick skeletal muscle (and after whom certain MS channels—the SAKs—are named) has comprehensively reivewed work from his lab [41–45]. I will focus mostly on material not dealt with previously; this is not an exhaustive summary but an attempt to highlight areas of progress and of uncertainty. Except for a preliminary report on the crustacean stretch receptor [14], MS channels in specialized mechanoreceptor cells have not been studied at the single channel level and will not be covered in this review, but discussions of mechanoelectrical transduction by hair cells, in light of what is known about SA channels, are available [23, 44].

MS Channels are Revealed by Their Currents

A list of MS channels according to phyletic group is provided in Table 2. These "channel observations" are based exclusively on current measurements (there are no binding assays for MS channels); except for the conventional voltage-clamp studies on ciliates [10, 63], the currents were observed at the single channel level. The protozoan cells are sophisticated in their mechanosensitivity, exhibiting two MS conductance mechanisms, one that hyperpolarizes (by virtue of its K^+ selectivity) and one that depolarizes the cell [30]. It is too early to know how these protozoan MS conductances are related to the two main classes of SA channels described for me-

Key Words ion channel · stretch-sensitive · mechanotransduction · single channel recording

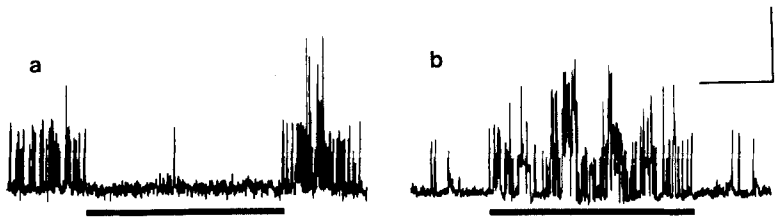

Fig. 1. Patch-clamp recordings of (*a*) SI and (*b*) SA channels from snail neurons. About −40 mmHg was applied to the patch during the periods indicated by the bars. Both types of channel are K^+-selective and are shown passing outward K^+ currents. Details of the recordings conditions can be found in ref. 38. Scale: (*a*) 1 pA, 2 sec; (*b*) 4 pA, 2 sec

Table 1. Channel nomenclature: The generic term MS (mechanosensitive)[a]

Subtypes of MS channels
1. Stretch-sensitive channels
i) SA: stretch-activated
SAAn (SA and predominantly permeable to anions, particularly chloride)
SACat (SA and permeable to most cations)
SAK (SA and predominantly permeable to potassium and analogs)
SANon (SA and nonselective or poorly selective between anions and cations)
SACa (SA and selective for Ca^{2+} and other divalents. Unlike the other stretch-sensitive channels listed here these are putative—they have not been observed at the single channel level)
ii) SI: stretch-inactivated
SIK (SI and predominantly permeable to potassium and analogs)
2. Displacement-sensitive channels (the putative transduction channels of hair cells as described in ref. 23)
3. Shear-stress-sensitive channels (putative; see ref. 40 and section on nonchannel MS membrane proteins in this article)

[a] MS conductance mechanisms in categories 2 and 3 will not be covered further here.

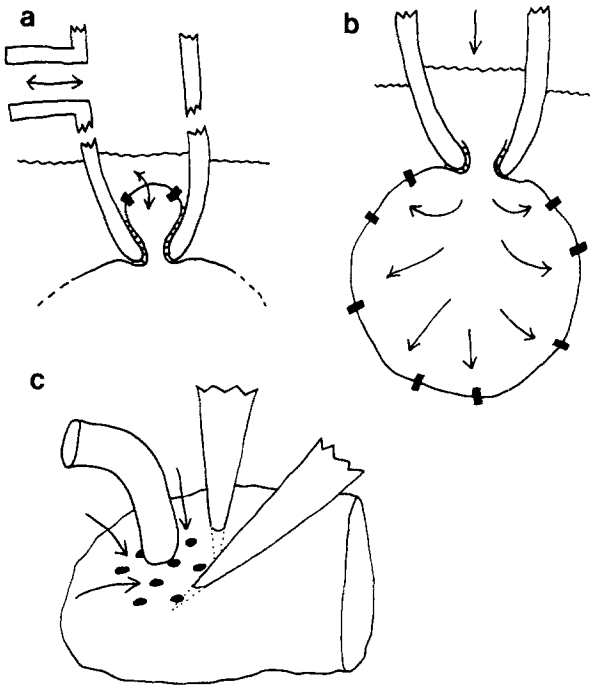

Fig. 2. Three methods for recording MS currents under voltage-clamp conditions. (*a*) Patch clamp, either excised or cell-attached. Mechanical stimulation is provided via a port on the electrode-holder assembly, to which monitored positive or negative pressure can be applied. The gigohm seal between membrane and recording pipette (suggested by the hatch lines) secures the membrane patch while it is deflected inward or outward according to the sign of the pressure. The magnitude of the applied pressure and the detailed geometry of pipette-plus-membrane determine the resulting membrane tension. (*b*) A patch electrode used in the whole-cell configuration. Ensemble currents are usually obtained, but for small cells, individual events may be resolved. Positive pressure, applied as for patch clamp, distends the cell. (*c*) Two microelectrode voltage clamp (current-injecting and voltage-monitoring electrodes penetrate the cell); a glass probe provides a macular stimulus to a region of membrane whose diameter is ill defined

tazoan forms. Among the metazoans, the major SA channels are a type permeable to both monovalent and divalent cations (SA "non-selective" cation [SACat] channels; the ciliate version may prefer divalents, but *see* ref. 30) and a type selectively permeable to K^+ (SAK channels).

In studying MS channels (Fig. 2), a number of voltage-clamp methods are used. At the single channel level, the patched membrane is stressed by positive or negative pressure. (It should be noted that when suction is used to promote gigaseal formation, it is transient; pressure is returned to atmospheric (760 mmHg) after the seal forms.) In whole-cell mode, mechanostimulation inflates the cell. A fine glass probe attached to a piezoelectric stylus provides the mechanical stimulus for most studies of MS currents in ciliates.

MS Channels Studied by Patch Clamp

For MS channels, questions of control of the mechanostimulus have the same import as do questions of space clamp and series resistance for volt-

Table 2. Mechanosensitive ion channels in various taxa

- Procaryotes
  - Bacteria
    - *Escherichia coli*, spheroplasts [31]*
- Eucaryotes
  - Fungi
    - Yeast (*Saccharomyces cerevisiae*) spheroplasts [19]*
  - Plants
    - *Nicotiani tobaccum*, protoplasts [15]†
    - *Vicia faba*, guard cell protoplasts [52] "depolarizing channel"
  - Animals
    - Protozoans
      - Ciliates (for review *see* 30; [63]‡§)
    - Molluscs
      - Pond snail heart cells [58]‡
      - Pond snail neuron, soma/growth cone [38, 56]‡¶
      - Terrestrial snail neurons [1]‡†
      - Marine gastropod (*Aplysia*) [1]‡
    - Arthropods
      - Crustacean stretch receptor neuron [14]§
      - Insects
        - Cockroach mechanosensory cells (A. French, *personal communication*)§
        - *Drosophila* muscle [68]‡
    - Tunicates
      - Ascidian oocyte [36]§
    - Vertebrates
      - Fish—blastula, loach embryo [32]‡
      - Amphibians
        - Skeletal muscle, frog [5]§
        - Oocytes, frog [33]§
        - Lens epithelium, frog [8]§
        - Choroid plexus, salamander [7]§
        - Kidney, proximal tubule, salamander [47]‡
        - Smooth muscle, stomach, frog [26]§
        - Erythrocytes, frog [65]** (*see also* [20]‡)
      - Birds
        - Chick skeletal muscle [17]§
        - Chick cardiac myocytes (F. Sachs, *personal communication*)§
      - Mammals
        - Ventricular myocytes, rat [9]§
        - Kidney, opossum cell line [62]*
        - Neuron, dorsal root ganglion, rat [64]**
        - Endothelial cells, pig aorta [28]§
        - Fibroblasts, human cell line [61]§
        - Mesangial cells, rat[a]**
        - Neuroblastoma [16]§
        - Astrocytes, rat [11]*¶§
        - Osteoblast-like cell line [12]§

The following symbols denote the type(s) of MS channels found in the listed preparation: * SANon, † SAAn, ‡ SAK, § SACat, ¶ SI, ** too little information available to specify.

[a] Craelius, W., El-Sharif, N., Palant, C.E. 1989. (*Submitted*).

age-gated channels. Ideally, one would wish to have a direct measure of the tension generated in a membrane during mechanostimulation. In practice, this is impossible. Instead, membrane tension is inferred from formulae which relate membrane geometry to applied force; it is usually assumed that the membrane is stimulated isotropically. Caution needs to be exercised. A cursory observation [6] indicated to us that patched membranes (Fig. 2*a*) are often neither close to the pipette tip nor hemispherical in shape as depicted and as originally described [50]. Patches, seen as far as 10 micrometers inside the pipette, were sometimes flaccid- or irregular-looking in the absence of suction. Using high resolution videomicroscopy, Sachs and co-workers (*personal communication; see also* 44–46) simultaneously recorded SA currents and membrane movements on excised patches. Patch behavior included travel of the sealed region along the pipette wall, pinching off of vesicles, and tethering (or even active tugging) by strands of cytoplasmic material. In more normal cases, pressure caused a flattened disc [45] of membrane to distend in the direction of applied pressure with no movement at the perimeter. The mechanical status of the membrane is, unfortunately, unknown in most experiments, since use of cell-attached patches or pipettes with optimal signal-to-noise characteristics (thick-walled, tapered, fire-polished, Sylgard-coated) precludes optical monitoring.

These shortcomings notwithstanding, the importance of the patch technique in revealing MS currents as discrete jumps can hardly be overstated. MS currents at the single channel level are readily distinguished from "membrane breakdown currents" seen in patches stressed by extreme voltage or mechanical disruption. The discovery of stretch-inactivated channels [38] (*see* Fig. 1*a*) makes it particularly hard to contend that MS currents are nonchannel leaks. The idea of ion-selective discrete-sized leak pathways in the bilayer which *close* with membrane stretch seems wildly improbable. A second advantage of the patch technique is the ease of applying a mechanical stimulus, at least for qualitative tests of stretch sensitivity. Excised patches allow one to rule out second messenger artifacts such as the activation of channels by Ca^{2+} (whose entry might be induced by a general disruption during stretch). For many of the channels listed in Table 2, mechanosensitivity has been demonstrated in cell-attached and excised patches. Both positive and negative pressures are effective in gating MS channels (for a SA channel, *see* [55]; for a SI channel, *see* [38]; for a recent exception to this generalization, *see* [11]), indicating that membrane curvature is not critical to MS gating. Single channel studies have revealed that SA channel activation is sigmoidal (*see* Fig. 3) with applied pressure, yielding curves for P_{op} *vs.* pressure that approximate the sigmoidal form that would be inferred from a Boltz-

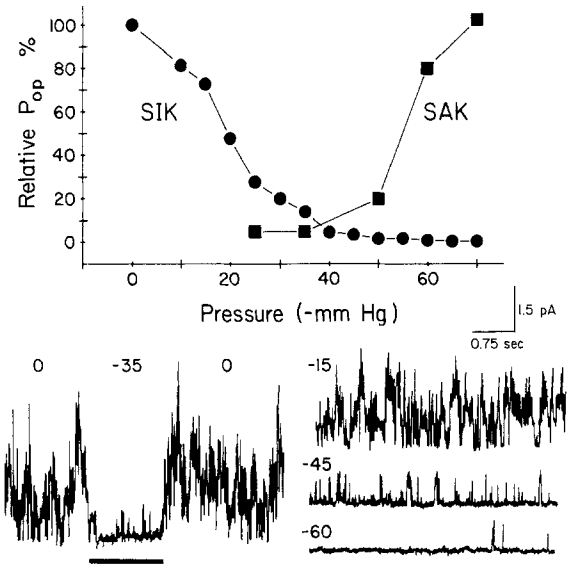

Fig. 3. Plots of the relative probability of being open for SIK and SAK channels from snail neuron (*see* ref. 38 for details). Raw data showing SIK channel activity at various pressures are given below

mann distribution of discrete open and closed channel states (e.g., [8]). It is noteworthy, and perhaps surprising, that in patches with several channels, the P_{op} *vs.* pressure curves do *not* assume a "staircase" form, as might be expected if (i) each channel had a unique sensitivity to stretch arising from the details of its coupling to elastic membrane elements or if (ii) various regions of the patched membrane experienced different tensions.

Kinetic analyses of single channel events have shown that stretch-dependent increases in P_{op} can largely be accounted for by decreases in the duration of a long closed time [17, 58, 36[1]]. This stretch-sensitive closed time is interpreted as being an interburst event [58]. Brief closed and open times (in the 1–2 msec time range) show little or no change with pressure, certainly not enough to explain many-fold changes in P_{op}. It is intriguing that this remains true for a SI channel; in this case, a long (interburst) closed time *increases* as P_{op} *falls* with increasing pressure [38]. The question of whether increasing burst length also contributes to increased P_{op} needs to be addressed, since the answer has implications for gating models. If both frequency and duration of bursts increases with tension over the entire P_{op} range, then at least two transitions (one "forward" and one "reverse") should be tension sensitive. To date, burst analyses of MS channel data [58, 32] have been inadequate to settle the issue. The stretch-induced increase in burst length reported recently for fish embryo SAK channels [32] does not necessarily reflect a stretch-sensitive burst-ending transition. Burst duration was analyzed only at zero pressure and at a near-saturating pressure, so burst length would inevitably appear to increase as individual bursts coalesce near maximal P_{op}.

[1] Brezden, B.L., Gardner, D.R. (*in preparation*).

MS Channels Seen in Whole-Cell Clamp

Distension of a cell under whole-cell clamp (Fig. 2*b*) is a means of mechanostimulation which was used to good effect in yeast cells [19]. Because standard whole-cell clamp causes washout of cytoplasm, MS currents so measured are analogous, from the biochemical standpoint, to those from excised patches. Membrane mechanics may, however, be more controlled and reproducible in whole cell than in a patch, because proportionally more membrane is remote from the supporting pipette rim. Gustin and co-workers [19] used capacitance measurements to estimate the membrane area (and hence diameter) of the clamped yeast spheroplasts of various sizes, and thereby could test whether P_{op} conformed to the predictions of Laplace's Law, $T = pd/4$ (where T = tension, p = applied pressure, d = diameter; applicable for an elastic thin-walled sphere) when the sheroplasts were subjected to pressure. Whereas there was no fixed dependence of P_{op} on pressure—the pressure at which P_{op} rose steeply varied inversely with cell diameter—there was a fixed dependence of P_{op} on the tension calculated from the Laplace relation. This elegant treatment provides the first experimental support for the generally-held assumption that MS channels are sensitive not to the pressure gradient, but to the membrane tension ("stretch") induced by pressure.

A somewhat surprising feature of the yeast SA channel is that it had an unexpectedly low conductance (36 pS) for a channel that distinguishes poorly between cations and anions. To help confirm that the same channel species does indeed carry anions and cations, the whole-cell clamp was exploited further: SA single channel events were obtained with only a sugar in the bath ("contaminant" electrolytes were sufficient to complete the circuit) but with CsCl inside the cell. When clamped to ±60 mV, the cell produced essentially identical-looking inward (Cl^- moving out of the cell) and outward (Cs^+ moving out of the cell) SA unitary currents. A further interesting trait of this channel is that it exhibits two approximately equally spaced open levels. A similar characteristic has recently been noted

in an astrocyte MS channel [3]. Large multiconductance Cl^- channels in excised snail neuron patches are sensitive to membrane stretch [1]. It would be worth testing for mechanosensitivity in "multibarrelled" channels [24], since large-area channel structures are predicted [17] to be effective at collecting mechanical energy.

MS Currents Seen by Conventional Voltage Clamp

Voltage-clamped membrane can be deformed with an external probe, as in the *Paramecium* experiments (Fig. 2*c*). One advantage of the probe is speed (a step stimulus with a risetime less than a millisecond can be produced (*see* [30]); by contrast, the fastest steps produced at a pipette tip have risetimes of tens of milliseconds [45]). A feature of probe stimulation is the maintenance of cytoplasmic integrity, important insofar as cytoskeletal elements affect MS gating [17] directly (i.e., as part of the transduction process) or indirectly (i.e., as series or parallel elastic elements). Using a probe on ciliate membrane, the latency between the onset of membrane deformation and the onset of the SA K^+ current is 1–9 msec; the current risetime is then about 5 msec [10]. The value of this post-latency risetime—measured following a step stimulus—is comparable to the predictions [44] of a kinetic scheme developed from SACat single channel data obtained at steady-state tensions.

Cellular Distribution of MS Channels

For some types of channels, density varies by several orders of magnitude, from "hot spots" of hundreds of channels per μm^2 (a patch pipette generally isolates 5–10 μm^2 of membrane) to areas essentially devoid of channels. The pattern correlates with function; density is high for acetylcholine channels at the neuromuscular junction and for Na^+ channels at nodes in myelinated nerve. MS channels display no such range of densities; they tend to be ubiquitous, occurring at uniform density (on the order of one per μm^2; [6, 36, 44]) and in every cell. Substantially higher densities, and certainly hot spots, have not been reported. Sachs calculates [42] that the ability of MS channels to collect mechanical energy would be optimal at a density of about 1.5 per μm^2, and offers this as an explanation for the apparently low density of MS channels (*see* [23]) in the exquisitely mechanosensitive hair cell. An underlying assumption in the calculation—that the P_{op} of a MS channel changes as the square of membrane tension rather than as tension—is, however, countered by Howard and co-workers [23].

The Flux of Ions through MS Channels at Uniform Low Density

Could MS channels at the observed densities support physiologically significant ion fluxes? "Ballpark" calculations based on single channel data and cellular parameters (e.g., size, input resistance, ion concentrations) suggest that they can. In molluscan muscle cells, a population of 1200 SAK channels could (assuming $P_{op} = 0.5$ and single channel current = 0.5 pA) deplete the cell of 1% of its K^+ in 1 sec [6]; SAK channels activated during hyposmotic swelling [57] may, therefore, contribute to swelling limitation [39]. Christensen [7] calculates that SACat channels in amphibian choroid plexus cells (present at about 0.2 per μm^2), if activated by hyposmotic swelling, could raise intracellular Ca^{2+} enough to stimulate the Ca^{2+}-activated K^+ channels which mediate regulatory volume decrease. For frog smooth muscle (with its high resting input resistance), it is estimated [26] that opening of a small fraction of the SACat channels would drive the membrane potential to a level that recruits voltage-gated Ca^{2+} channels. Ca^{2+} entering directly through the SACat channels plus that entering via the voltage-activated channels could then explain the phenomenon of stretch-induced contraction exhibited by these mechanically excitable cells. For ascidian oocytes, calculations indicate that recruitment of less than 1% of the SACat channels could bring the membrane to threshold for activation of voltage-dependent Na^+ and Ca^{2+} channels [36].

Measurements of the permeation of SACat channels by Ca^{2+} [7, 8, 28, 36, 66] have also led to conclusions that the channels could act as a significant route for entry of extracellular Ca^{2+}. Interpolating from permeation properties at high Ca^{2+} concentrations, it is estimated [66] that in a physiological mixture (150 mM Na^+, 1 mM Ca^{2+}) the current carried at −60 mV by Ca^{2+} through a SACat channel could approach 0.1 pA. Fortunately, an antagonist to the SACat channel has been found, so that the reliance on arithmetic to gauge the importance of ion fluxes through MS channels during cellular events may lessen. Gadolinium, the antagonist, is a trivalent lanthanide which in plants interferes with geo- and thigmotropic responses; the suggestion was made [34] that it might act via plant SA channels [15]. Testing gadolinium on animal cells (frog oocytes and myocytes), Yang and Sachs [66] found that the lanthanide reversibly blocks SACat channels. It also blocks the nonselective

yeast MS channels [19], but is ineffective on astrocyte SAK channels [66]. Gadolinium block, which is essentially complete at 10 μM, results from several actions [66]: a diffusion-controlled binding reaction to a site in the permeation pathway for Na^+ and Ca^{2+} (the site is located outside the membrane electric field), a reduction in single channel current resulting from screening of surface charge and, unexpectedly, a reduction in the rate of opening. Though gadolinium will not be an unproblematic tool, it will be far more useful in physiological studies of SACat channels than is quinidine in physiological studies of SAK channels [6, 39] because, unlike quinidine, gadolinium is membrane impermeant and is fully effective in the sub-millimolar range. Neither gadolinium nor quinidine appear to affect mechanotransduction in the MS channels they block. The alkaloid, curare, and its analog gallamine, inhibit MS currents in the ciliate, *Stentor*, in the 1–10 μM range [63]; investigation of this action at the single channel level in SACat channels seems to be warranted.

Establishing the physiological significance of MS currents with single channel recordings alone is difficult. Ensemble recordings (e.g., [40] made in conjunction with patch studies (e.g., [19, 36]) of MS currents in cages where a blocker can be used to link the two levels of observation should soon produce new insights.

Some MS Channels are Inhomogeneously Distributed

Nonuniform distributions of MS channels have been characterized in only two cell types. The MS currents in *Paramecium* [30], which participate in directional control of ciliary beating, show an end-to-end polarization; at the anterior of the cell, stimulation elicits a Ca^{2+} current, whereas posterior stimulation elicits a K^+ current. Machemer and Deitmer [30] discuss, but reject, the suggestion [51] that two channel types (SACa and SAK) are uniformly distributed but have different stretch sensitivities. Instead, they conclude that there is a "gradient-type" distribution of the two MS channels, established at early stages of cell division. Single channel work on snail neurons [38] has demonstrated that different types of MS channel can occur in close proximity, making the idea of an overlapping distribution in *Paramecium* entirely plausible.

Another report of nonuniformity of MS channel distribution is that of SACat channels in human fibroblasts grown in nonconfluent tissue culture [61]. SACat channels, which responded reliably to −20 mmHg, were found preferentially on the cell borders, with densities ranging from 0–18 channels per patch. Continual movements take place at the cell margin; it is suggested that the channels function in stress measurement.

Cultured snail neurons can be patched in two regions with distinctly different mechanical properties: the mechanically-quiescent (one assumes) cell body, and the mechanically-active growth cones [56]. SAK and SIK channels occur in both cellular regions, with no discernible differences in channel density, stretch sensitivity or permeability characteristics. While this does not rule out the possibility that the channels subserve motility at the growth cone and some other role in the soma, it makes it all the more imperative to find ways of directly testing hypotheses about channel function. A further issue regarding the SIK channel distribution is that, unlike SAK channels, SIK channels are not observed in every patch. Because neurons are idiosyncratic, it may be that SIK channels are expressed only in particular cells or types of cells. Their sporadic occurrence may, however, have a more banal explanation, namely that they are present but inactivated by residual tension in the pipette tip ([38] and *see* below). The issue is not trivial; where SIK channels occur, they would constitute a major component of the resting conductance. Moreover, where they coexist with SAK channels, the partnership yields a "notch-filter" ([38]; *see* Fig. 3). The intermediate membrane tensions at which K^+ permeability is minimal should facilitate excitability and therefore voltage-dependent Ca^{2+} entry.

Epithelial cells, which by definition are functionally polarized, contain MS channels; the channels have been reported variously in apical and basolateral membranes (lens epithelium, apical membrane, [8]; choroid plexus, ventricular surface, [7]; renal proximal tubule, basolateral membrane [47]), but information is lacking on whether they are confined to one membrane surface only in a given preparation.

MS Channels are Absent in Early Stages of Some Cells

Questions of distribution are temporal as well as spatial. Do cells always have functional MS channels or do they acquire them in the course of their life histories? Are MS channels absent in all newly elaborated membrane? To date, answers to these questions are "yes" to the first, "no" to the second. In two distantly related embryonic myotube preparations (*Drosophila* and chick), it is reported that the SA channel activity is initially absent (<12 hr in culture for the insect, <3 days in culture for

chick), whereas in the differentiated mytotubes, the channels are reliably present [44, 68]. The *Drosophila* SAK channels found in cultured embryonic muscle [68] appear to be homologous to those we have studied in nerve and muscle cells of adult snails; SAK channel activity was normal in the adult neurons as soon as recordings could be made following isolation, i.e., within 0.3 hr [56]. In addition to showing that SAK channels are not merely a cultured-cell phenomenon, the presence of SAK channels in freshly isolated adult neurons renders it less likely that their absence from early *Drosophila* myocytes is an isolation artifact. Sachs [44] speculates that what prevents SA channel activity in early myocytes is the cells' lack of spectrin. Why should spectrin matter? Because this submembranous cytoskeletal protein may be used, via its links to integral membrane proteins, in providing mechanical advantage to stretch stiff MS channel structures [17, 44]. If this conjecture is correct and spectrin-like elements are critical to MS gating, these elements must be rapidly organized as new membrane is added to growing neurites; growth cone patches from rearborizing snail neurons invariably showed SAK channel activity [56].

Are Physiological Membrane Tensions Large Enough to Gate MS Channels?

The relevance of MS channels to the life of a cell hinges on whether membranes actually experience tension changes in the range which would change the channels' P_{op}. Unfortunately, we know exceedingly little about the magnitude of cellular membrane tensions. (Many difficulties would be circumvented if an enterprising chemist could act on Daedalus' tongue-in-cheek suggestion and concoct a "Loadlacquer" [25] for cell physiologists—a nontoxic tensiometric membrane dye.) Because of our ignorance about absolute tensions of membranes *in situ*, it is important to glean what we can from the extreme cases—tensions that rupture the membrane and tensions near zero. Knowing how MS channels behave at the extremes may help justify some hand-waving for the intermediate tensions.

Membrane Rupture

When applying suction to a patch pipette, −200 mmHg is usually excessive and ruptures the membrane. By comparison, most SA channels are reported to be maximally activated at −10 to −120 mmHg. During cytokinesis, rupturing tensions will be generated along sites of fission, and lower tensions will be generated elsewhere. In cell-attached recordings of embryonic fish cells undergoing cytokinesis, cyclic changes in SAK channel P_{op} have been recorded [32]. Since patched membrane is mechanically isolated from extra-patch membrane, the patch would not "feel" (laterally) membrane tension associated with cell cleavage. The patch must therefore be perturbed in the perpendicular direction, presumably by cytoplasmic material coupled to it. Tensions produced in this manner in patched membrane (which should not exceed those in extra-patch membrane) were sufficient to drive 5- to 20-fold changes in P_{op}. Changes in SAK channel P_{op} provide a simple explanation for oscillations of membrane potential during the cell cycle [32], an explanation made all the more convincing by independent evidence [67] for periodic changes in membrane tension in cleaving eggs. The possibility that the changing P_{op} is driven by a cycling chemical has not, however, been rigorously ruled out.

Motility

As we have pointed out [38], the tensions generated in patch electrodes to activate SA channels are of the same order as those measured [21] in migrating fibroblasts. It becomes difficult therefore, to imagine that fibroblast SACat channels [61] would be entirely quiescent when the cell migrates along a substrate.

Growth cone tensions have not been measured, but the existence of tension-producing mechanical forces in the advancing neurite can be inferred by phenomena like the towing by growth cones of their cell body over a substrate (under culture conditions in which the growth cone adheres more strongly than the cell body) [4]. Bray [4] views tension as central to growth cone behavior: "(m)echanical tension allows the growth cone to test a path by 'integrating' the multiple adhesive interactions it experiences on its surface, whether . . . specific biochemical interactions between proteins or nonspecific physical adhesions. All can be converted to the common currency of mechanical force, which can subsequently be consolidated into growth." With two types of MS channels now known to occur in growth cones [38, 56], it will be necessary either to show that they are unaffected by changes in growth cone tension or to incorporate them into the picture.

Hyposmotic Shock (HOS)

A HOS (or an intracellular osmotic perturbation resulting from a catabolic process like glycogen →

glucose → lactate, or peptide → amino acids) of 1 mosmol is, by definition, equivalent to −18 mmHg for a cell that is a perfect osmometer. Knowing that −200 mmHg ruptures membranes, and that water permeability greatly exceeds solute permeability for most membranes, one would expect a mere 10–15 mosmol HOS to be fatal. Cells are not, however, osmometric balloons. They can expand membrane folds or incorporate new membrane when subjected to HOS and many cells exhibit volume-induced increases in permeability to various ions. These permeability changes could reflect measures taken to lose osmolytes, to signal changing membrane tension or both. The question of whether SA channels are activated under conditions of HOS [37] is clearly worth examining.

Christensen [7] showed (i) that amphibian choroid plexus cells contain SA channels that could bring Ca^{2+} into the cell, and (ii) that Ca^{2+}-activated K^+ channels are recruited during HOS, and he thus concluded that SA channels were activated by the swelling-induced increase in membrane tension; swelling-induced SA channel activity was not, however, demonstrated directly. To accurately quantify HOS-induced activation of MS channels is not easy. A patched membrane is isolated laterally from tension generated by cell swelling. If the patch compliance matched that of the rest of the cell, this would be irrelevant, but the geometry (curvature) of the patch should reduce its compliance. Moreover, a patch formed several microns within the pipette will be effectively in series with a mechanical resistance. Thus, SA channels isolated in a patch may only whisper when SA channels in the rest of a swelling cell are shouting. On the positive side, if HOS *can* activate even channels in a patch, the argument that swelling is too weak a perturbation to affect MS channels in the cell proper become less tenable. Four single channel channel reports have now appeared showing such HOS-induced activity of MS channels.

Opossum kidney cells were exposed to 60% HOS (i.e., 60% of normal osmolarity) while the channel activity in a cell-attached patch was monitored [62]. In a given patch, an identical number of channels (up to 6 per patch) could be activated either by HOS or by direct mechanical stress (i.e., suction). The characteristics of the HOS- and suction-activated channels were identical: a nonselective channel of small conductance, reminiscent of the MS channel of yeast [19]. Sackin [48], using a 50% HOS, obtained similar results for a frog kidney SAK channel. Though the experimental conditions prevented the cells from exhibiting their normal volume regulatory decrease, HOS induced SAK channel activity whenever the cells swelled to >130% of their original volume. Falke and Misler [16], using 65–79% HOS on neuroblastoma cells, observed that SACat channels were activated with a time course similar to cell swelling and that, as expected if the stimulus is mechanical, activity subsided during the regulatory volume decrease phase. Snail neurons subjected to 50% HOS hyperpolarized and showed reversible swelling-related increases in SAK channel activity in 35% of patches [57]. The physiological relevance of HOS-induced channel activation in all these experiments remains to be seen. The use of unphysiologically large HOS optimizes the chance of seeing changes in MS channel activity, but leaves open the question of whether small-scale swelling of cells (about 10%) would, as suggested by the calculations of Sackin [48], activate SA channels.

To the consternation to those using frog oocytes as a channel expression system, the cells are well-endowed with SACat channels [33]. What are the channels for? They may well play a role in embryogenesis, but an additional possibility that has been suggested [19] is that the SA channel help the egg cope with a dauntingly large HOS—the quick trip from frog to pond, an event that is all the more traumatic because Laplace's Law dictates a large tension change for this large diameter cell. Swelling-induced activation of the SACat channels would produce a net release of K^+ and Na^+ and entry of Ca^{2+} to signal further regulatory processes. With a blocker now available for SACat channels [66], it should become possible to pursue such physiological speculations. It is curious that freshwater fish eggs have SAK channels [32] rather than the SACat channels found in amphibian [33] and ascidian [36] eggs.

Mechanoreceptors

We know that mechanostimuli delivered via a patch electrode are able to activate SA channels, but are uncertain how these stimuli compare with the perturbations to which cells are subject. Since mechanoreceptors should be *at least* as sensitive as ordinary cells to mechanostimuli, it would be useful to know how SA channels of mechanoreceptors respond under patch-clamp conditions. The modified mechanosensory dendrites of crustacean stretch receptors remain inaccessible to patch electrodes, but recordings have been made from the close-by cell body of these neurons [14]. The SACat-like MS channels appear to be sensitive to pressure in the range −30–−40 mmHg, in keeping with MS channels of many nonspecialist cells. Perhaps, then, the tensions generated in patch-clamp experiments of ordinary cells are not "pathologically" large. A ca-

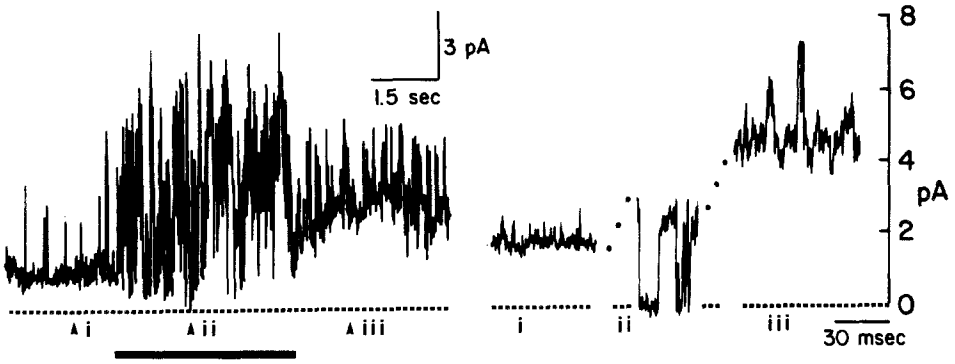

Fig. 4. A recording from a snail neuron patch with several SIK and SAK channels. Pre-stretch "noise" is due to spontaneously active SIK channels and an occasional SAK channel (note the zero current level given by the broken line). During stretch, SAK channel activity dominates; after stretch, SIK channels dominate. Arrowheads *i–iii* indicate sections of record expanded at right. The ~3 pA events (*ii*) rising from the zero-current level are SAK channel currents. SIK channel transitions create ~0.5 pA blips (*i*) in the noisy pre-stretch trace, but discrete events cannot be distinguished. Recording conditions: cell-attached with normal saline in the pipette, membrane potential at +70 mV (recording conditions as in ref. 38; W. Sigurdson and C.E.M., *unpublished*)

veat should accompany this line of reasoning, however; though cell bodies from snail ganglia invariably possess MS (SAK) channels [56], one would not argue *a priori* that they are representative of channels in mechanosensory endings. By the same token, MS channels in the crayfish stretch-receptor cell body may or may not be representative of those in the modified dendrites. It would be helpful to compare mechanosensory and nonmechanosensory crayfish neurons.

Effects on SI Channels Should be Hard to Avoid

In exploring whether cells are likely to encounter tensions sufficiently large to gate MS channels, we have concentrated on SA channels. What of SI channels? Such channels have recently been found in two preparations: snail neurons [38] and mammalian astrocytes [3, 11] (the glial cell channels differ in being inactivated by suction but not by positive pipette pressure). SI channels turn off at tensions below those needed to activate SA channels. We found that snail neuron SI channels were always at least half-inactivated by −15 mmHg [38]. Is it conceivable that they are *not* affected by normal cellular mechanics? With threshold tensions so close to zero tension, could inactivation always be avoided? With only two SI channels known, the question could be deemed less than pressing. But SI channels can easily be overlooked (as they were initially by us) and therefore may be more widespread than is presently realized. If, as mentioned earlier, residual pipette tension inactivates the SI channels, they will go unnoticed unless the tension is countered [38]. A second problem, illustrated in Fig. 4 is that activity of small conductance SI channels is easily dismissed as "noisy baseline" if SA channels are activated during cursory observations of the effect of stretch. Observing SAK channel turn-on in Fig. 4 is straightforward; SIK channel turn-off is more difficult, but can be inferred from the rare, random intervals (during stretch) when no SAK channels are open (e.g., Fig. 4*ii*), since, at these points, current goes not to pre- or post-stretch levels, but to zero. Upon release, the overall activity falls (SAK channels turning off) but the "baseline" shifts up (SIK channels reactivating; such rebound activation beyond the prestimulus level is not uncommon, as in Fig. 3). Less dramatically, one often sees, in snail neurons, "small background channels" whose activity, happily (or so we used to feel), subsides as the seal improves. Hindsight suggests they are probably SI channels responding to slight (uncontrolled) increases in patch tension. Residual pipette tension is unexceptional (*see* [7, 32, 47, 38], all of which indicate that nominal-zero is not necessarily true-zero tension). Considering the potential physiological consequences of SI channels—they constitute a resting permeability component sensitive to perturbations smaller than those needed to activate SA channels—it would be well not to overlook them. An intriguing possibility, for instance, is that anterior MS currents of ciliates might be due not to SACa channels, as is always assumed, but to SIK channels in parallel with stretch-independent Ca^{2+} channels. This could be tested by looking for a decrease in membrane noise upon mechanostimulation.

In snail neuron growth cones, it has recently been demonstrated [35] that mechanostimulation

(e.g., puffing saline from a pipette) induces an increase in intracellular Ca^{2+} and subsequently an altered growth pattern. It is tantalizing to speculate that suppression of SIK channel activity might facilitate Ca^{2+} entry [38]. The flow from a perfusion pipette can activate SAK channels in an excised patch [58] and can slightly hyperpolarize neuronal cell bodies [57]. Even weaker mechanical stimuli of growth cones should therefore suffice to inactivate SIK channels.

Transduction Models

What kind of coupling with its immediate environment could make a 2-state channel (open and closed conformations) capable of reporting on the mechanical status of the membrane—how could mechanical stimulation cause it to change its P_{op}? The channel is ensconced in a bilayer which has a surface tension, but the surface tension of the bilayer is effectively a fixed property, not subject to change by reasonable biomechanical events. The relevant "membrane tension," for mechanoreception is not therefore the (interfacial) surface tension of the membrane, but the elastic, tensile stress to which the membrane is subject. The material experiencing these mechanical forces is not, in all likelihood, the bilayer alone, but some combination of bilayer plus membranous cytoskeletal network and extracellular matrix. It is to this poorly specified entity that we will be referring in using the term "membrane."

In response to a mechanical stimulus, the change in free energy of either of the two channel conformations is given by the work done to achieve the elastic deformation. The elastic work can be equated to a free energy (ΔG) whose value is obtained by integrating the force (which we will call a tension, T) over the distance of the deformation ($\Delta G = \int T dx$). Assuming that this elastic deformation obeys Hooke's law, force $= T = K_a(x - x_o)$, where K_a is the spring constant and $(x - x_o)$ is the distance through which the spring is stretched. On integration, this yields $\Delta G = T^2/2K_a$. Note that if, instead of a linear spring, one is dealing with an elastic plane, the spring constant becomes an area elasticity and there are changes in area to consider instead of changes in length. Either way, this line of reasoning predicts a squared dependence of mechanosensitive transition rates on applied force, as in the model proposed by Guharay and Sachs [17, 44] rather than the first-order dependence of the proposed displacement-sensitive mechanism of hair cells [23].

But we are dealing with two rather than one idealized elastic objects (open and closed conformations of the channel; I assume that the elasticity of the rest of the membrane is, to a first approximation, unaffected by the thermal transitions of the channels, and that the open and closed channel elastic constants exceed that of the rest of the membrane and are consequently "rate-limiting"). Hence (as pointed out by Harold Lecar, *personal communication*), the free energy of any conformation as a function of applied tension will be given by

$$G_i = G_i^0 + T^2/2K_i \tag{1}$$

where G_i^0 is some tension-independent intrinsic free energy of the conformation and K_i is an elastic coefficient for the particular conformation. Thus, at any given tension, the free energy *difference* between the states is given by

$$\Delta G_{(op-c)} = \Delta G^0 + (T^2/2K_{op} - T^2/2K_c) \tag{2}$$

where $\Delta G^0 = G_{op}^0 - G_c^0$. In the special case in which the open conformation was indistensible (infinitely stiff, i.e., $K_{op} \to \infty$), note that

$$\Delta G_{(op-c)} = \Delta G^0 - T^2/2K_c. \tag{3}$$

If the equilibrium distribution for the open and closed states obeys a Boltzmann distribution, then

$$P_{op}/P_c = \exp(-\Delta G_{(op-c)}/kT^\circ) \tag{4}$$

(k is the Boltzmann constant and T° is temperature) and the probability of being in the open state obeys

$$P_{op} = [1 + \exp(\Delta G_{(op-c)}/kT^\circ)]^{-1} \tag{5}$$

since $P_{op} + P_c = 1$. Moreover,

$$\begin{aligned} P_{op}/P_c &= \exp(-\Delta G^0/kT^\circ)\exp(-T^2/2K_{eff}kT^\circ) \\ &= B_0 \exp(-\theta T^2) \end{aligned} \tag{6}$$

where the constant, $B_0 = \exp(-\Delta G^0/kT^\circ)$ and $\theta = 1/(2K_{eff}kT^\circ)$, and $1/K_{eff} = 1/K_{op} - 1/K_c$, noting again that for a rigid open conformation ($K_{op} \to \infty$) $K_{eff} \to K_c$.

Why this insistence on the stiffness of the open state? How might this be important in building an efficient mechanoreceptor? To answer, it is useful to consider for a moment the classical view of the 2-state voltage-dependent channel [13]. In this model, the opening and closing rates are biased symmetrically (in an equal and opposite manner) by the transmembrane potential. A family of free energy barrier diagrams for the two states (as P_{op} goes from near 0 to near 1 in response to various voltages) shows a fixed barrier height, but the depth of both

wells change with the changing field. At $P_{op} = 0.5$ the wells are, by definition, of the same depth. Now apply this picture to a SA channel—effectively, make stretch increase the opening rate and decrease the closing rate). Gating structures in channels in the closed conformation would be *distended* by an applied (stretching) force and gates in the open channel conformation ought, by analogy, to be *compressed*. Although it is possible to imagine a system of molecular levers which could compress some gating region of the channel while producing a net stretch in the rest of the open channel, this is a rather contrived picture. If there were evidence that the closing rate (or some equivalent burst-ending rate) decreased with increased tension, one would want to look at this idea seriously, but there is, as yet, no compelling evidence for more than one stretch-sensitive transition. It is easier, instead, to imagine that the closed conformation is distensible (and therefore expands under stretch, making it easier for thermal events to nudge the channel into the open configuration) and that the open configuration is rigid. In this case, the barrier diagram describing the open and closed conformations would have a fixed barrier height, a fixed well level for the open conformation, and the well depth for the closed conformation would vary. Near $P_{op} = 0$, at near-zero tension, the closed conformation has a lower free energy than the open state (giving ΔG^0, above), at $P_{op} = 0.5$ the well levels are the same and near $P_{op} = 1$, the closed conformation energy exceeds that of the open conformation.

ΔG^0 depends on the intrinsic internal (potential) energy difference (open *vs.* closed) and on the differences of the statistical weighting of the two conformations among their molecular substates (the entropic term in the free energy difference). With this in mind, let us continue to think about a rigid open state. The intrinsic free energy difference between a distensible closed state and a rigid open state is likely to be largely entropic, that is, the "soft" (distensible) closed state would achieve its lower free energy by virtue of its many accessible microstates. The rigid open state should admittedly, have a relatively low potential energy (by virtue, say, of favorable charge-charge interactions which stiffen it against distension in the plane of stretch), but when both entropic effects and potential energy are accounted for, the closed state comes out lower. Hence, the channel is overwhelmingly in the closed conformation in the absence of an additional source of energy, and it is able to "report" on the availability of elastic energy in the membrane by opening and passing current.

This scheme would yield a SA channel if the closed state were distensible (and probably smaller in area) and a SI channel if the open state were distensible (and probably smaller in area) (Fig. 5). (I allude to membrane-planar area here, and do not mean to imply that the "volume" of the more distensible state should be smaller). Although the MS channels discussed in this review, like most channels, have not just two states, but several open and closed states [17, 36, 58], the general theme applies with the understanding that certain transitions (between, e.g., two closed states) take the channel closer to an opening transition and others take it further from an opening transition.

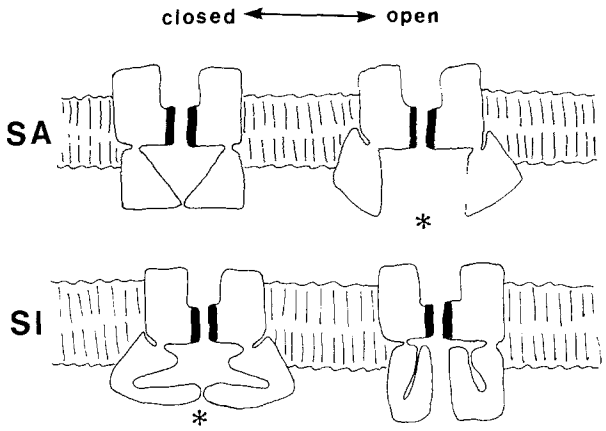

Fig. 5. A simplified elastic transduction model for SA and SI channels. The selectivity filter (black region) is unaffected by membrane tension. At any given tension, the channel is at thermal equilibrium between closed and open states, but the closed ↔ open fluctuation rates are a function of membrane tension (*see* text for discussion of asymmetry of tension effects). The channel state favored in the absence of stretch is likely to be more distensible, and, on common-sense grounds, smaller, whereas the larger, stiffer state (asterisks) becomes more probable when the membrane has been stretched. This yields a SA channel (small, soft closed state) and a SI channel (small, soft open state). To recall the contributions of mechanical and thermal factors in "gating" the channel, think of equilibrium at a tension that yields $P_{op} = 0.5$; here, *transitions "against" the prevailing tension* occur as frequently as transitions "with" the prevailing tension. Individual transitions of (stochastic) channels are by definition, thermally induced; when tension biases the probability of a certain state-to-state conformation change, the collective activity (many individual transitions) is referred to as "a mechanically induced transition." The scheme is depicted without energy-collecting cytoskeletal elements connected to the channels (*see* ref. 42), but a means of elastic coupling, either intrinsic or extrinsic to the bilayer, is assumed for this elastic model. In reality, MS channels may operate in a viscoelastic medium, but no provision for this has been made in the model. (As drawn, the model suggests that SI and SA channels of the same selectivity could have different access pathways and therefore might have different conductances and sensitivities to channel blockers, as is the case for SIK and SAK channels [38])

Kinetic analysis of the SACat channel data (assuming a C ↔ C ↔ C ↔ O scheme to connect three

closed states and one open state) over a range of pressures suggested [17] that only one transition (the forward transition between the left-most closed states) was stretch sensitive. Retaining the notion of a 2-state model (allowed because the equilibrium situation, not the equilibration process is under consideration), Guharay and Sachs [17] used a Boltzmann relation of the following form

$$P_{op} = [1 + (\alpha/\beta_0) \exp(\theta p^2)]^{-1} P_{max} \quad (7)$$

in which θ is a stretch sensitivity and tension is assumed to be linearly related to applied pressure, p, according to Laplace's Law. The ratio α/β_0 ($=j$ in the models below), sets the probability that the channel is open at zero pressure; α is the fixed closing rate, β_0 is the tension-dependent opening rate at zero tension (*see also* [8]). P_{max} is 1 for a two-state channel, but realistically would be <1 for a channel with several closed states, congruent with observations (e.g., [58]) that at saturation, channel noise does not approach zero, as it would for a two-state channel.

It has not been shown that models based on the square of tension are, in fact, better predictors of P_{op} than models with a first-order tension (e.g., [23]). The membrane may, for example, be non-Hookean (like a spring operating near its elastic limit) (*see also* ref. 29) or mechanical stress may favor a movement like the flipping of a tethered dipole (for a voltage-sensitive channel, this leads to a free energy which is first order with respect to the applied force [13]). For the bacterial channel [31], the first power of tension was adequate. As an exercise, W. Sigurdson and I, in collaboration with R. Horn, analyzed four data sets using a statistical method [22] for discriminating between non-nested models. The data were from chick muscle [17] and frog lens SACat channels [8], and from snail heart [58] and snail neuron (*unpublished*) SAK channels. Given the small number of points (i.e., the co-ordinates: P_{op}, p) per data set, the method of choice was regression analysis of resampled data (1000 resampled data sets were created from each original set, and the distributions of the log error ratios were examined). Keeping the general form of Guharay and Sachs' equation, we compared two models (model II is also equivalent to that developed above)

$$\text{I: } P_{op} = [1 + j \exp(\theta_1 p)]^{-1}$$
$$\text{II: } P_{op} = [1 + j \exp(\theta_2 p^2)]^{-1}$$

and found (i) that the original data sets could be fit by either expression and (ii) that model I was statistically better for muscle SACat channels and neuron SAK channel, whereas model II was better for the lens SACat channel and heart SAK channels. Note that (like Cooper and co-workers [8]), we implicitly used $P_{max} = 1$ though it is evident from the raw data that a smaller value would be appropriate. Admittedly, the physical meaning of θ_1, with units of inverse pressure, is less clear than that of θ_2 (which is discussed in ref. 17). Nevertheless, this exercise convinced us that getting an acceptable fit is disconcertingly easy, even with "wrong" models. Showing that a fit to a model of interest is better than fits to other appropriate models therefore becomes an important next step. Data sets obtained under conditions in which the patch geometry is monitored would aid considerably in deciding between various predictors of P_{op} (unless someone suggests that tension should be raised to a power between 1 and 2!).

Mechanosensitivity May Sometimes be a Red Herring

While it is incontrovertible that SA channels *can* be activated by membrane deformation, stretch may not always be what the channels "listen to" physiologically. Major confounding factors to rule out are transmembrane voltage and ligands. Bacterial SA channels recorded from spheroplasts are more voltage sensitive [31] than other MS channels which have been examined at the single channel level. If, however, the bacterial MS channels are situated in the outer membrane in parallel with open porin channels, the MS channels may seldom experience voltage excursions. Effects of voltage on most other MS channels (Table 3) are relatively minor. Given the near-zero P_{op} reported for SACat and SAK channels in the absence of applied pressure, voltage sensitivity equal to or less than that reported for chick muscle SACat channels [18] (i.e., P_{op} increases e-fold per 45 mV) would be relatively ineffectual. If, however, tension were biased so that a non-zero P_{op} was normal, the added effect of voltage could be significant. The question of bias may be important for the ciliate, *Stentor*, whose MS channels are steeply voltage dependent, but reveal this characteristic only during mechanostimulation [63].

Of the multitude of chemical stimuli that might regulate MS channel gating, only intracellular Ca^{2+} (SACat channel [17] and SAK channel [58]) and extracellular protons (SACat channel, [18]) have been examined. It has been shown that MS channels in excised patches retain their ability to respond to membrane stretch at vanishingly small concentrations of these ions and that millimolar Ca^{2+} does not

Table 3. Summary of the effects of membrane potential on MS channels

Channel	Ref.	Comments
SACat (chick muscle)	18	At physiological pH (7.4), P_{op} increases e-fold per 45 mV.
SACat (frog smooth muscle)	26	Overall P_{op} is voltage-independent from $V_m = -130$---30 mV, but increases thereafter (not more than e-fold per 75 mV between $V_m = -25$ and $+50$, and continuing at a steeper slope for larger, nonphysiological depolarizations).
SACat (neuroblastoma)	16	Voltage-independent at ± 30 mV from rest.
SACat (frog oocyte)	33	P_{op} increases with depolarization. Noise analysis at -70 mV and $+30$ mV shows that the relative contribution of the slower of the two components of the spectrum (a sum of Lorentzians) increases with depolarization.
SAK (fish embryo)	32	$\tau_{open} = 1.5$ msec at -40 mV $= 2.1$ msec at $+40$ mV $\tau_{closed} = 62$ msec at -40 mV $= 57$ msec at $+40$ mV
SAK (*Drosophila* muscle)	68	Voltage-independent or slight decrease in P_{op} with depolarization (tested at $V_m = -40, 0, +40, +80$ mV).
SAK (snail neuron)	57	P_{op} increases not more than e-fold per 143 mV between $V_m = -60$ mV and $+30$ mV. (In the nonphysiological range beyond this, voltage sensitivity increases to e-fold per 19 mV).
SAK (ciliate, posterior)	30	Decay constant of receptor current decreases e-fold per 110 mV (from $V_m = -115 - 0$ mV). Risetime, over same range, e-fold per 410 mV.
SANon (*E. coli*)	31	P_{op} increases e-fold per 15 mV.
SACa (*Stentor*)	63	P_{op} increases e-fold per 12.6 mV. The effect of V_m is secondary to the transduction process (i.e., not sufficient in itself to activate the MS channel).

in itself activate the channels. This allows one to be reasonably confident, when monitoring physiologically induced activity in cell-attached patches (e.g., [16, 32]) that what is perceived to be mechanical gating is not in fact Ca^{2+} or proton gating. However, there may be channels that can choose from a "menu" of stimulants/inactivators: intracellular Ca^{2+}, voltage, stretch, assorted ligands.

Unpalatable though it may be, there is also the possibility that, in spite of their mechanosensitivity, some SA channels are in reality "2-methyl-whatever" channels. With no change in tension, the channels might turn on, given the right second messenger. Sensitivity to stretch might be an epiphenomenon, an accident, perhaps, of attachment (for reasons of density control) to a spectrin-like submembranous network. A precedent exists for channel-cytoskeletal attachments. Sodium channels in rat brain are tied down by ankyrin and spectrin [60]. Anion channels ("band 3") of erythrocytes are connected to spectrin [50]. In neurons, the density of glutamate receptors appears to be regulated by the spectrin-like protein, fodrin [59]. It is, unfortunately, unknown whether connections to the cytoskeleton render these channels stretch sensitive.

A molluscan K^+ channel well-known for its control by neuromodulators is the S-channel, a serotonin- and FMRFamide-regulated K^+ channel of mechanosensory neurons in the marine gastropod, *Aplysia* [2]. Preliminary single-channel work on unidentified *Aplysia* neurons showed that S-channel-like currents could be activated by suction [1]. Snail SAK channels share a variety of properties with S-channels (density, permeation characteristics, voltage insensitivity and pharmacological properties [6, 54, 58, and *unpublished results*]); it will be interesting, therefore, to determine (i) whether the *Aplysia* S-channel is stretch sensitive and (ii) whether snail SAK channels are susceptible to neuromodulators or second messengers.

Nonchannel MS Proteins Need to be Considered

The shear stress-activated conductance of endothelial cells [40] suggests another wrinkle in relation to second messengers and MS channels. Fluid shear over the whole cell induced an inwardly rectifying K^+ conductance, but at the single-channel level no evidence could be obtained to demonstrate direct

stretch-activation of the conductance. Perhaps shear does not act directly on the channel, but on a membrane protein which controls a second messenger, thereby up-regulating the channel. Considering that tension can selectively affect particular channels, yielding either positive or negative stretch-sensitivity [17, 36, 38, 68] while having no affect on others, it is plausible that classes of membrane protein other than channels also have members sensitive to tension.

Conclusions

In the half-decade since MS channels were first observed, much has been achieved in working out the "natural history" of the channels. They are now seldom shrugged off as artifact; instead, because they are so widespread, one begins to see them as standard membrane machinery. We know that they come in several varieties (selectivity differs, as does the polarity and sensitivity of the tension effect), but we remain largely ignorant about why they are there and how they work. An understanding of transduction awaits (i) better cell and molecular biological understanding of the channels (are they really tied into the cytoskeleton?) and (ii) kinetic analysis under precisely controlled and widely varied conditions. A major stumbling block for physiological work has been the lack of specific blockers for the channels. For the SACat channel, that may now change. Another encouraging development, which may eventually open up molecular genetic routes for studying MS channels, is the finding of SAK channels in *Drosophila*; the next trick will be to figure out what behavioral quirks to look for in a fly suffering for lack of wildtype SAKs.

I am much indebted to Harold Lecar for sharing his insights about things mechanical (from statistical to celestial, with emphasis transductional). I am also grateful to Dick Horn for his generous crunching of numbers, to Wade Sigurdson for hours of discussion and to Fred Sachs for his all-purpose enthusiasm. My research is supported by the Natural Sciences and Engineering Research Council of Canada.

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