Molecular Basis of Mechanotransduction in Living Cells

OWEN P. HAMILL AND BORIS MARTINAC

Physiology and Biophysics, University Of Texas Medical Branch, Galveston, Texas; and
Department of Pharmacology, University Of Western Australia, Nedlands, West Australia

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Hamill, Owen P., and Boris Martinac. Molecular Basis of Mechanotransduction in Living Cells. *Physiol Rev* 81: 685–740, 2001.—The simplest cell-like structure, the lipid bilayer vesicle, can respond to mechanical deformation by elastic membrane dilation/thinning and curvature changes. When a protein is inserted in the lipid bilayer, an energetic cost may arise because of hydrophobic mismatch between the protein and bilayer. Localized changes in bilayer thickness and curvature may compensate for this mismatch. The peptides alamethicin and gramicidin and the bacterial membrane protein MscL form mechanically gated (MG) channels when inserted in lipid bilayers. Their mechanosensitivity may arise because channel opening is associated with a change in the protein’s membrane-occupied area, its hydrophobic mismatch with the bilayer, excluded water volume, or a combination of these effects. As a consequence, bilayer dilation/thinning or changes in local membrane curvature may shift the equilibrium between channel conformations. Recent evidence indicates that MG channels in specific animal cell types (e.g., *Xenopus* oocytes) are also gated directly by bilayer tension. However, animal cells lack the rigid cell wall that protects bacteria and plants cells from excessive expansion of their bilayer. Instead, a cortical cytoskeleton (CSK) provides a structural framework that allows the animal cell to maintain a stable excess membrane area (i.e., for its volume occupied by a sphere) in the form of membrane folds, ruffles, and microvilli. This excess membrane provides an immediate membrane reserve that may protect the bilayer from sudden changes in bilayer tension. Contractile elements within the CSK may locally slacken or tighten bilayer tension to regulate mechanosensitivity, whereas membrane blebbing and tight seal patch formation, by using up membrane reserves, may increase membrane mechanosensitivity. In specific cases, extracellular and/or CSK proteins (i.e., tethers) may transmit mechanical forces to the process (e.g., hair cell MG channels, MS intracellular Ca\(^{2+}\) release, and transmitter release) without increasing tension in the lipid bilayer.

I. INTRODUCTION

Cells experience a wide variety of mechanical stimuli ranging from thermal molecular agitation to potentially destructive osmotic pressure gradients. Therefore, from the onset, living organisms faced a basic dilemma in their evolution. As a first priority, they required mechanisms that would protect their delicate cell membrane from potentially damaging mechanical stimuli. However, to interact with their changing mechanical environment (e.g., during feeding, escaping, or mating), they needed mechanosensitivity. Different organisms have solved the dilemma by different strategies. Bacteria and plants evolved a rigid cell wall that protects their plasma membrane from excessive dilation. However, with this strategy they sacrificed not only cell deformability but also mechanosensitivity. In contrast, animals have adopted strategies that protect their cell membrane while preserving a high degree of cell deformability and mechanosensitivity.

The first mechanosensitive (MS) processes may have evolved as backup mechanisms for cell protection. For example, a large nonspecific membrane pore activated by osmotic swelling will release the cell’s contents and thereby reduce intracellular pressure and membrane tension. Similarly, tension-sensitive fusion of intracellular membrane vesicles with the cell membrane will act to reduce bilayer tension. These basic mechanisms of mechanically gated (MG) channels and MS exocytosis may have been subsequently refined to participate in cell signaling. For example, MG channels and/or MS transmitter release are implicated in a myriad of physiological processes, including touch and pain sensation (46, 292, 403), hearing and vestibular function (148, 190), blood pressure control (45, 61), salt and fluid balance (32), micturition (36), tissue growth (98), cell volume regulation (301, 308, 430), and turgor control (147, 265). Furthermore, abnormalities in these mechanisms may contribute to neuronal (93) and muscular degeneration (116), cardiac arrhythmia (86, 117, 162), hypertension (224), arteriosclerosis (90), and glaucoma (282).

The external mechanical forces that dominate a cell vary depending on its size and relationship with other cells. For example, unicellular organisms like *Escherichia coli* are constantly jostled by the forces of Brownian motion that tend to keep them in suspension. In contrast, multicellular organisms require specific MS mechanisms that constantly adjust their position in response to gravity. Furthermore, specific cells, depending on their location within an organism and association with ancillary structures, may be selectively exposed to specific forms of mechanical stimuli, including steady indentations, high-frequency vibrations, osmotic pressure gradients, and hemodynamic pressure and fluid shear stresses. All external stimuli act on top of a dynamic background of various internally generated forces (e.g., arising from hydrostatic pressure, cytoskeletal polymerization, and molecular motors) that are important in determining cell shape, growth, mobility, and adhesion (15, 201). To monitor and respond selectively to these different forces most likely requires multiple, parallel signaling pathways, with each pathway designed to extract specific information regarding the “relevant stimulus” while filtering out irrelevant stimuli.

Over the last 20 years, the molecular nature of specific MS membrane processes has been identified. These include MG membrane ion channels (156, 265, 286, 350) and MS receptors (53, 320), enzymes (241, 270), intracellular Ca\(^{2+}\) release (204) and transmitter release (63).
cause each of these elements or processes may interact with one another, as well as with other non-MS elements, difficulties can arise in distinguishing mechanisms that are directly or indirectly affected by mechanical forces. Furthermore, given that a single cell may express multiple mechanotransducers, a challenge can arise in determining which transducer mediates a specific MS function (i.e., cause and effect relations). A notable example is vertebrate tactile sensation where the basic distinction between physical and chemical mechanisms of mechanotransduction has yet to be made (cf. Refs. 135, 292). In principle, one should be able to identify the mechanotransducer by comparing its specific properties (i.e., sensitivity, kinetics, and pharmacology) with those of the MS function.

A. Basic Requirement for Mechanosensitivity

For a membrane protein to be directly MS, it must be sensitive to a membrane property that changes with mechanical deformation. For the specific case of a simple two-state channel, a shift in the equilibrium between closed and open channel conformations may be caused by changes in bilayer tension, thickness, or local curvature or by direct “tugging” on the protein by cytoskeletal or extracellular tethers. Therefore, a fundamental issue in mechanotransduction is the identification of the membrane parameter that actually confers mechanosensitivity on the membrane protein or process.

B. Strategy and Scope of This Review

The membrane of most animal cells is a composite structure of extracellular (EC), bilayer, and cytoskeletal (CSK) layers. Because of its integrated nature, any externally applied force produces varying tensions and strains in multiple elements within the three layers (200). For this reason, it becomes problematic in identifying a single membrane property that may be directly involved in the mechanotransduction process. To overcome this problem, we adopt a hierarchical approach and consider a variety of membrane preparations, progressing from the simple artificial bilayer vesicle to increasingly more complex cells (i.e., from bacteria to animal cells). The rationale for this approach is that if characteristics of a particular mechanism can be identified (i.e., “finger-printed”) in a simple system, one should be better positioned to recognize its operation in more complex systems. Our approach would seem justified by the reoccurring theme in evolution in which basic mechanisms that first evolved in prokaryotes are conserved and refined to carry out more diverse and specialized functions in eukaryotes. However, some processes such as exocytosis/endocytosis and release of Ca\(^{2+}\) from intracellular membrane stores are unique to eukaryotes (54), and therefore, their mechanosensitivity must reflect more recently acquired mechanisms of mechanotransduction.

II. LIPID BILAYER STRUCTURE AND ITS RESPONSE TO MECHANICAL DEFORMATION

Because the bilayer is the core structure around which all other membrane components are arranged, it is critical to understand its molecular packing and how this packing may change under steady state and dynamic mechanical deformation. For example, intrinsic delays or relaxations in the response of the bilayer to deformation may be reflected in the functional dynamics (i.e., frequency response and adaptive behavior) of MG channel activities. The bilayer is composed of lipid molecules that form two monolayers stabilized by van der Waals forces and the “hydrophobic” effect between the “hidden” acyl lipid chains. In addition, water molecules surrounding each lipid headgroup form hydrogen bonds that further stabilize the bilayer (180). Water molecules also penetrate deeper into the bilayer, hopping between acyl chain packing defects, such as trans-gauche kinks. For example, it is estimated that ~4,000 water molecules pass a single phospholipid per second compared with 1 Na\(^+\) every 70 h (85). At reduced temperatures, lipid bilayers undergo a liquid-gel phase transition in which the acyl chain packing becomes more ordered (187a). Furthermore, bilayers made up of more than one phospholipid can undergo lateral phase separations (138, 221). In the case of cell membranes, it is generally assumed that their complex lipid and cholesterol makeup ensure a highly fluid state at physiological temperatures. However, recent studies indicate preferential packing of sphingolipids and cholesterol into floating platforms or rafts of lipid that can be isolated as detergent-insoluble membrane complexes (309a). Although the occurrence of such phase separations will complicate bilayer and cell membrane mechanics, their specific effects have been little studied.

In principle, it is possible to study the mechanics of planar lipid bilayers, typically formed by painting a film of phospholipid over a hole in a plastic barrier, (e.g., see Ref. 112). However, such studies are complicated by the presence of a torus of disordered lipid that can act as a lipid reservoir for formation of new bilayer (336, 433a). In contrast, the lipid vesicle is a more “cell-like” structure in that it is a closed system that has its volume set by the osmotic activity of the aqueous environment. How the lipid vesicle responds to mechanical deformation depends on both extrinsic (e.g., size and geometry) as well as intrinsic properties (i.e., material elastic properties) (see Refs. 107, 109). For example, a deflated vesicle filled with volumes insufficient to form a sphere is highly de-
formable but somewhat unstable with a tendency to spontaneously “bud” or vesiculate. In contrast, a vesicle inflated by osmotic or hydrostatic forces into a sphere is stable but shows limited deformability in that with further inflation (i.e., 2–4%) it either ruptures or under specific circumstances forms pores (452). Transient pore formation by releasing intravesicular pressure will preserve vesicle structure and thus may have served as an inbuilt protection mechanism for primordial cells before they evolved protein mechanisms.

With the assumption that the lipid bilayer behaves as an elastic solid, its intrinsic mechanical properties can be characterized by four elasticity constants (or moduli) that describe the response of a unit area of bilayer to compression, expansion, bending, and extension (108, 109). The larger the moduli, the greater the resistance to that form of deformation. Elastic deformations are directly proportional to and follow instantaneously the application and removal of external forces. In comparison, viscoelastic or plastic deformations show time dependence, and one has to take into account the different viscosity coefficients for each type of deformation (108). The elastic moduli of bilayer vesicles and human red blood cells (RBCs) have typically been measured with the micropipette aspiration technique (106). A critical feature of this technique is that there is minimal membrane adherence to the pipette to ensure reversible and unimpeded movement of the aspirated portion of the membrane within the pipette. Under these circumstances, the membrane tensions developed during aspiration can be assumed isotropic throughout the vesicle, with the membrane protrusion drawn into the pipette serving as an amplified measurement of membrane area changes. In contrast, in patch-clamp recording, the membrane adheres tightly to the walls of the pipette (153). As long as the membrane-glass adhesion is not disrupted (i.e., the patch boundary remains constant), tension changes will be restricted to the “free” membrane area that spans the inside of the pipette (see sect. viIA).

A. Membrane Compression

An early study based on the effects of pressure on the lipid bilayer phase transition demonstrated that hydrostatic pressures up to \(-1 \times 10^7\) N/m\(^2\) (i.e., 100 atmospheres) did not significantly alter the bilayer density change associated with the phase transition (378). Based on this result, Evans and Hochmuth (108) estimated the bilayer compressibility modulus was between 10\(^9\) and 10\(^10\) N/m\(^2\), similar to that of most “incompressible” fluids. A subsequent study based on X-ray diffraction analysis of osmotically stressed liposomes gave similar estimates (275, see also Ref. 322). Although higher compressive forces (i.e., 500–1,500 atm) have since been shown to increase acyl chain packing density and squeeze water out of pure phospholipid bilayer, these effects are minimized in bilayers that include cholesterol (16, 334a). Thus one may assume that the bilayer of the cell membrane is volumetrically incompressible and will maintain a constant density during the mechanical deformations encountered under physiological conditions. As indicated below, the resistance to volume compression is at least an order of magnitude larger than the resistance to bilayer thickness and area changes.

B. Membrane Area Expansion/Thinning

The tight lateral packing of lipid molecules in the bilayer underlies its extremely low ion permeability and relatively low water permeability (85). However, this feature also contributes to the bilayer’s resistance to area expansion. This is because even slight additional separation of lipid head groups (i.e., \(-2\%\)) will allow more water to enter between the acyl chains and destabilize the hydrophobic cohesive structure. Aspiration of spherical vesicles indicates a simple linear relation between membrane tension (\(t\)) and the relative area expansion of the bilayer

\[
t = K_A \cdot \Delta A/A_0
\]

where \(\Delta A\) is the increase in surface area, \(A_0\) is the original area, and \(K_A\) is the area expansion modulus (108). Typical values of \(K_A\) range between 10\(^2\) and 10\(^3\) mN/m depending on the cholesterol content of the bilayer, while lytic tensions range between 3 and 30 mN/m, consistent with bilayers only being able to be expanded 2–4% before rupture (108, 296, 298). With the assumption of a bilayer compressibility modulus of 10\(^9\) N/m\(^2\), the bilayer is at least 10-fold more compressible in area than in volume (given a \(K_A\) of 200 mN/m divided by 3 nm for the bilayer thickness). Thus, at near lytic tensions, the area may increase by \(-4\%\), but the volume by \(<0.4\%\) so that the thickness will decrease by \(3.6\%\). Thus any fractional change in area should be accompanied by a proportional change in membrane thickness (\(h\)) so that

\[
\Delta A/A_0 = -\Delta h/h_0
\]

where \(h_0\) is the unstressed membrane thickness and the expansion and thickness moduli are related according to \(K_A = K_h \cdot h_0\) (108). Estimates of \(K_h\) based on capacitance measurements of bilayer thickness changes during electrocompression indicate values of \(-2 \times 10^7\) N/m\(^2\) (5), which would predict a \(K_A\) of \(-70\) mN/m assuming a bilayer membrane thickness of 3 nm (108).

Bilayer vesicles generated from membranes of RBC (183, 295, 296) and skeletal muscle (298) give values of \(K_A\) of around 500 mN/m, similar to the \(K_A\) measured for
osmotically swollen (i.e., spherical) RBCs (106, 183). This agreement has been taken to indicate that the CSK of the RBC does not limit the elastic expansion of the bilayer (108). In studies of other cell types in which significantly lower values of \( K_A \) have been reported (e.g., 2–20 mN/m, e.g., see Ref. 187), it is not clear that elastic membrane expansion at constant area was being measured, since the cells were not preswollen to reduce excess membrane surface area. Interestingly, in the case of RBCs, it has been reported that \( K_A \) values vary by \( \pm 40\% \) with voltage changes of \( \pm 200 \text{ mV} \) (210, 211). Although the mechanism of this polarity-sensitive \( K_A \) effect remains unknown, it may reflect electric forces acting on packing of the highly asymmetrical lipid bilayer of the RBC (458). In this case, it will be interesting to examine the voltage effects on RBCs that have lost their phospholipid asymmetry due to lipid scrambling (152, 458).

C. Membrane Bending/Curvature

The resistance of the bilayer to bending arises because of differential expansion or compression of the two monolayers within the bilayer and will depend on how tightly the two halves of the bilayer are coupled (i.e., degree of interdigitation between the acyl chains). If there is no coupling so that the two monolayers can rapidly slide past one another, there will be little resistance to bending. Bending rigidity is also dependent on the spontaneous curvature of the bilayer, which depends on the lipid composition and area of each monolayer (see sect. m) as well as the coupling of the CSK to the bilayer (91, 431). In the specific case of a bilayer sealed tightly to the walls of the patch pipette, the bending rigidity of the membrane patch should be increased because the attachment of the outer monolayer to the pipette walls will restrict its movement relative to the inner monolayer. Although the estimated bending modulus of the bilayer (\( K_B \sim 10^{-10} \text{ N} \cdot \text{m} \)) indicates that bending resistance is significantly less than resistance to expansion (108), it is the bending rigidity that determines the shape of the lipid vesicle, its elastic response to membrane dimpling, and the amplitude of thermally induced fluctuations in the vesicle (452a; 276a and references therein).

D. Membrane Extension/Shear

Above the phase transition temperature, the bilayer has unrestricted internal fluidity and displays negligible surface shear rigidity so that it flows like a fluid in response to shear or extension. Below the phase transition, the shear rigidity increases along with hydrocarbon chain order (108). Furthermore, bilayers that undergo lipid phase separations may be expected to show heterogeneities in shear rigidity (138). Similarly, the existence of lipid rafts in cell membranes (369a) may result in differential rates of lipid flow in response to shear. However, more important is the cortical CSK that by providing the fluid bilayer with a solid support significantly increases the shear rigidity of the cell membrane and thereby allows elastic responses to membrane extension (see sect. vii). For example, the human RBC has an elastic shear modulus of \( \sim 10^{-2} \text{ mN/m} \) and can recover rapidly from large extensions. However, after treatments [e.g., >48°C or increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) that disrupt the cortical CSK, the shear modulus is so diminished the RBC undergoes spontaneous fragmentation (e.g., see Fig. 8 in Ref. 284; Ref. 152).

E. Viscous Properties and Dynamic Response of Bilayer Vesicles

Elastic solids respond instantaneously to deformation. However, a bilayer vesicle cannot be deformed instantaneously because of the inertia of surrounding water movement and in specific cases possibly due to the viscous properties (e.g., bending viscosity) of the bilayer itself (24). For bilayer expansion, hydrodynamic drag rather than expansion viscosity is most likely rate limiting. For example, based on fluorescence polarization measurements of the bilayer hydrocarbon interior, the expansion viscosity (\( \nu_{A} \)) of the bilayer has been estimated to be \( 10^{-10} \text{ N} \cdot \text{s/m} \) (i.e., comparable to a 10-Å-thick layer of olive oil, Ref. 108). In this case, the time constant for area relaxation (\( \tau_{A} \)) will be \( 10^{-9} \text{ s} \) according to the relation \( \tau_{A} = \nu_{A}/K_A \) and assuming a \( K_A \) of \( 10^2 \text{ mN/m} \) (108). How does this value compare with experimentally measured kinetics of bilayer expansion? Clearly such measurements are limited by current methods. For example, even relatively sophisticated pressure-clamp techniques can only give pressure steps with a rise time of \( \sim 1 \text{ ms} \) (272, 273), and these cause membrane patch movements (i.e., expansion) and MG channel activation with millisecond latencies (448). In comparison, voltage steps (i.e., with a rise time \( <1 \mu\text{s} \)) applied to outer hair cells cause membrane patch expansions of \( \sim 1\% \) with a \( \tau \) of \( 10 \mu\text{s} \) (119a). The discrepancy may reflect membrane damping by the fluid movement in the pipette and/or the viscous drag of the cortical CSK. The relative contributions may be separated by measurements on blebbed (i.e., CSK-disrupted) hair cells. In terms of shear relaxation, the negligibly small shear modulus of the fluid bilayer should allow even faster intrinsic kinetics (i.e., \( <10^{-9} \text{ s} \)). The shear relaxation of cell membranes may be rate limited by the larger shear viscosity of the underlying CSK. In the specific case of bilayer bending, the interfacial drag between the two monolayers may be sufficiently large (i.e., \( 10^5 \text{ N} \cdot \text{s/m}^3 \)) to slow membrane bending and shape recov-
Evidence of slow bending kinetics may be reflected in the relaxation of fine membrane tethers drawn from lipid vesicles (181) and possibly the adaptation of MG channel activity in liposome membrane patches (see sect. VIII E).

In summary, the mechanical equilibrium of a lipid vesicle is established by the balance of external forces applied to the membrane (i.e., expansion and bending) opposed by the action of membrane tension and the bending rigidity. In considering deformation-sensitive membrane parameters that might influence membrane protein conformational changes, it is often overlooked that dilation of the elastic bilayer (i.e., increasing the area occupied by lipid molecules) should be accompanied by a proportional decrease in bilayer thickness (i.e., assuming volume incompressibility). In addition, and as described below, factors that affect spontaneous membrane curvature and bending rigidity may influence protein conformational changes. In terms of bilayer dynamics, that rate of membrane deformations involving bilayer expansion and extension may be damped by the hydrodynamics of the adjacent water compartments. However, membrane bending and relaxation may be rate limited by viscous drag between the monolayers.

III. MECHANICAL DEFORMATION OF THE BILAYER BY MEMBRANE PROTEIN INSERTION

The next level of complexity that can be considered is how insertion of membrane proteins may mechanically distort the bilayer and, in turn, how mechanically induced bilayer distortions may influence protein conformational changes. The central idea to bilayer-protein interaction is that the hydrophobic thickness of the bilayer immediately adjacent to the membrane protein will tend to match the length of the protein's hydrophobic exterior (Fig. 1; Refs. 290, 291). This may be expected to occur because any uncompensated mismatch will add a high energetic cost by exposing hydrophobic groups to water. Because proteins are relatively rigid, whereas lipid hydrocarbon chains are flexible, the condition of hydrophobic matching can be achieved by stretching, squashing, and/or tilting of the lipid chains (172, 193). Recent direct evidence supporting protein-induced changes in lipid organization comes from the demonstration that hydrophobic α-helical peptides, including gramicidin A, can change a bilayer into a nonlamellar structure, with this transition dependent on the degree of hydrophobic mismatch (217). Furthermore, insertion of peptides, including alamethicin, into bilayers causes a concentration-dependent thinning of the bilayer as measured by lamellar X-ray diffraction (173).

One of the consequences of the hydrophobic mismatch idea is that proteins will tend to surround themselves with lipids of matching size and shape so that the mechanical strain on the bilayer will be minimized (82, 111, 125, 140, 352). Furthermore, if the lipid composition of the membrane can be altered, one might expect to see shifts in protein conformational changes. Evidence supporting these ideas has come from studies of the effects of foreign phospholipids and lipophilic agents on MG channel activities (53, 251, 253). For example, the opposite effects observed with some lipophilic agents on gramicidin and N-methyl-D-aspartate (NMDA) channel kinetics may be explained by differences in lipid shape, as defined by the ratio of head group size (H) to the acyl tail area (T), on the localized curvature of the bilayer (53, 253). Lipids with \( H = T \) will tend to favor neutral curvature, lipids with \( H > T \) will favor positive curvature, and lipids with \( T > H \)

FIG. 1. A membrane protein in changing conformation also undergoes changes in hydrophobic mismatch with the lipid bilayer. A: positive hydrophobic mismatch in which the protein promotes local positive curvature in the lipid bilayer. B: a protein conformation that promotes local negative curvature. C: neutral mismatch in which the protein does not distort the bilayer. [Modified from Fattal and Ben-Shaul (111).]
will favor negative curvature. As described below, the effects of lipids of different geometry on MG channel gating provided the initial clue that changes in membrane thickness and/or local curvature may underlie one mechanism of MG channel gating (136, 251). The importance of the surrounding lipids on protein function may also be reflected in the enzyme-regulated (i.e., flippase and translocase) asymmetrical distribution of phospholipid in each monolayer, which is lost with scramblase activation (458).

Figure 1 considers the specific case of a membrane protein that has three stable conformational states, each with different types of hydrophobic mismatch with the bilayer (i.e., A positive, B negative, and C neutral). Insertion of foreign lipids or membrane thinning by altering the energetic cost of membrane deformation should cause shifts in the distribution among these conformations (e.g., membrane thinning would favor B). However, effects such as ligand binding, phosphorylation, or membrane polarization may cause shifts independent of hydrophobic mismatch. In this case, complex interactions may arise between mechanical and other forms of stimuli.

IV. SIMPLE PEPTIDES THAT FORM MECHANICALLY GATED CHANNELS

Alamethicin and gramicidin are the best-characterized membrane channels in terms of their biophysics (i.e., conductance and gating), structure-activity relations, and modeling of open-closed channel conformations (6, 47, 355, 426). Therefore, the demonstration that both channels display mechanosensitivity in pure lipid bilayers, similar to prokaryotic MG channels, has provided the opportunity to analyze possible underlying molecular mechanisms in extremely well-defined simple systems.

A. Alamethicin

Alamethicin is a 20-amino acid peptide that forms voltage-gated multi-conductance state channels in lipid bilayers (47, 355). The most commonly evoked model to explain channel formation is a "barrel-stave" model in which each stave of the barrel is formed by a single α-helical monomer. To explain the voltage sensitivity of alamethicin, two main classes of mechanisms have been proposed (47). In one, the channel exists as a preaggregate of subunits, and a voltage-dependent conformational change results in channel formation. In the other, alamethicin exists predominantly as monomers and voltage-dependent insertion (or partitioning) with subsequent aggregation that leads to channel formation. Because evidence exists supporting both mechanisms (47), analysis of the mechanosensitivity of alamethicin channel gating was used in an attempt to discriminate between the two mechanisms (313). Initially, Opsahl and Webb (313) demonstrated in patch-clamped bilayers (i.e., using the "tip-dip" method) that increased membrane tension increased the probability of the channel occupying higher conductance states. Their quantitative analysis of the relation between the state of occupation and applied membrane tension (t) was interpreted in terms of the work done in channel opening W = t · ΔA, where the switching between the adjacent states involves an increase in membrane occupied area of the channel complex (ΔA) of ~1.2 ± 0.10 nm². Based on these area changes, they proposed that the mechanosensitivity of switching between different conductance states could be explained by a model involving two tension-sensitive steps. Step 1 involved insertion or partitioning of an additional monomer (cross-sectional area ~0.8 nm²) into the channel bilayer. Step 2 involved the subsequent association of the inserted monomer with the existing channel aggregate resulting in an increase in pore area (~0.4 nm²). In favoring the subunit-recruitment model, Opsahl and Webb (313) pointed out that the observed area changes were most likely too large to be compatible with a model involving rearrangement (expansion) of the monomers within a fixed aggregate (115a). Furthermore, their observation that the free energy difference between closed and open channel conformations varied linearly with tension confirmed that first-order area changes were responsible for the mechanosensitivity, while second-order (quadratic) effects due to compliance changes in channel states were not significant (69, 349). An increase in pore area of 0.4 nm² would give an increase in pore volume of ~1.3 nm³ assuming a pore length of 3 nm. This volume change is at least of the same order predicted based on osmotic experiments that indicate channel switching involves uptake of up to 3 nm³ of water (421). Opsahl and Webb (313) demonstrated equivalent tension sensitivity for the three lowest adjacent conductance states in bilayers of fixed composition. It will be interesting to see if these same states as well as the higher states generated in bilayers with high curvature (214) display the same tension sensitivity as might be expected from their model. Finally, Opsahl and Webb (313) did not consider the free energy contribution on changing the environment of one face of the recruited alamethicin subunit from that low dielectric of the lipid bilayer to the high dielectric of the aqueous pore. As described later for a bacterial MG channel, the large free energy change associated with channel opening may arise from the energetic cost of exposing pore-lining, hydrophobic residues to water in the open pore (443, see sect. IV).
related to hydrophobic coupling between the peptide and bilayer.

B. Gramicidin

Gramicidin is a 15-amino acid peptide that also forms channels in bilayers. Although a simple molecule, it exhibits two different folding motifs: a double helix and a helical dimer. This polymorphism in structure is manifest in solution, in bilayers, and in the solid state (426). Although both folding motifs may form transmembrane pores or channels, there is substantial evidence that the most frequently observed channel arises through the dimerization reaction between two nonconducting monomers that insert into each monolayer as α-helices (306). The length of the gramicidin dimer exterior is 2.2 nm, which can be compared with ~3 nm for a phopholipid bilayer and 3.2 nm for the long axis of alamethicin (115a). As illustrated in Figure 1B, because of gramicidin’s negative mismatch, the bilayer hydrophobic core will tend to be compressed (i.e., seen as a negative curvature) to match the channel’s hydrophobic exterior surface.

Several groups have previously reported apparent tension sensitivity in the gating of gramicidin channels (101, 297, 341). However, interpretation of these early studies was complicated because channels were studied in black lipid bilayers where changes in tension remain undefined and in some cases membrane thickness as well as tension was altered. In a more recent study, the pipette aspiration technique was used to increase tension in bilayer vesicles (136, see sect. ii). With this technique it was demonstrated that tension increased the rate of gramicidin channel formation (2- to 4-fold) and, to a lesser extent, the average channel lifetime (136). Note this increase in lifetime was opposite to (2- to 4-fold) and, to a lesser extent, the average channel lifetime was altered. In a more recent study, the pipette aspiration technique was used to increase tension in bilayer vesicles (136, see sect. ii). With this technique it was demonstrated that tension increased the rate of gramicidin channel formation (2- to 4-fold) and, to a lesser extent, the average channel lifetime (136).

For gramicidin, there is little difference in the membrane area occupied by monomers and dimers that might explain the tension sensitivity of channel gating. Instead, Goulian et al. (136) proposed that increased tension, by causing bilayer thinning (see sect. iiB), improved the hydrophobic coupling between the bilayer and the dimer, thereby reducing the membrane deformation energy associated with channel formation and increasing the activation energy associated with channel dissociation. The smaller effect of tension on rates of channel dissociation compared with rates of formation were shown to be consistent with the smaller displacement (~0.1 nm) of monomers necessary for dissociation compared with the larger mismatch (i.e., ~0.4 nm) between the dimer and bilayer. In contrast to the linear dependence of free energy change of alamethicin channel switching with tension (313), gramicidin displayed a quadratic dependence (136), possibly reflecting the elastic properties of membrane deformation (252). According to Nielsen et al. (300), if a protein conformational change involves an increase in the hydrophobic mismatch from 0.1 to 0.13 nm, there will be a 10-fold shift in the equilibrium distribution of protein conformations due to these elastic properties of the membrane.

In addition to the effects of membrane tension, a variety of other experimental maneuvers have been shown to alter gramicidin channel gating. These effects were also interpreted as arising through changes in membrane deformation energy. The treatments include incorporation of membrane lipids, detergents, and cholesterol that tend to alter the membrane local curvature, thereby either stabilizing the channel, in the case of agents that promote positive curvature, or destabilizing the channel, in the case of agents that promote negative curvature (251, 253). Similarly, conditions such as elevated Ca2+ that decrease electrostatic energy of the bilayer by screening surface charge are proposed to promote channel dissociation by a mechanism involving changes in the membrane local curvature or thickness (254). Note that although the gramicidin channel is axially symmetrical, its sensitivity to the sign of local curvature of the adjacent lipid is expected in terms of the mismatch model (see Fig. 1). One would also predict that changes in membrane voltage, by altering membrane thickness through electroconstriction (5), would also alter membrane deformation energy and thereby gramicidin channel gating. However, voltage-sensitive gating of gramicidin has not been reported (6).

In summary, increased bilayer tension promotes dimerization of gramicidin and higher conductance states of alamethicin. It may be that different mechanisms underlie the mechanosensitivity of the two channels. For alamethicin, a subunit recruitment model has been favored over the fixed aggregate model. However, there is evidence that indicates the switching between alamethicin conductance states is dependent on lipid composition, similar to gramicidin except that the lipids that promote gramicidin dimerization favor lower conductance states of alamethicin (214, 251). Because other evidence indicates that membrane deformation energy may be the major driving force for the alamethicin insertion transition (see Ref. 171), the hydrophobic mismatch model may also contribute to the mechanosensitivity of alamethicin. Finally, the existence of polymorphic structures, ambiguities, and unresolved issues with such simple channel-forming peptides is a useful reminder of the complications that lie ahead for modeling larger and more complicated channel proteins as described in the next section.

V. Structure of Prokaryotic Cells

The discovery of Archaea (formerly achaebacteria) has clearly shown that the old prokaryote/eukaryote dichotomy is obsolete by largely oversimplifying diversity
of prokaryotic microbes in all its aspects including prokaryotic cell envelopes (436). Nevertheless, from the perspective of the MG channel gating mechanism, bacterial as well as archaeal cells may be considered the next level of complexity after a bilayer vesicle. The cytoplasmic membrane of bacteria is a fragile structure composed of phospholipids and proteins enclosed by a cell wall (i.e., outer membrane) that provides a strong, rigid structural component able to withstand the osmotic pressures caused by the intracellular concentration of various osmoticants in the cell (423). Without the mechanical support of the cell wall, a bacterial cell would behave as a tiny dialysis bag that would take up water from the environment, swell, and burst. Bacterial cell walls (with the exception of the mycoplasma) have a structural component called peptidoglycan that provides the rigidity necessary to maintain cell integrity. The major building blocks of peptidoglycan are N-acetylg glucosamine and N-acetylmuramic acid that are unique to bacterial cells. On the basis of the Gram stain, a differential staining technique invented in 1884 by Christian Gram, bacteria can be divided into two large groups: Gram positive and Gram negative, which differ in the peptidoglycan content of their cell wall (i.e., ~5 times larger in Gram-positive cells) (Fig. 2A) than in Gram-negative cells (see Fig. 2B). In addition, Gram-negative bacteria have a second chemically distinct outer membrane attached to the peptidoglycan layer on its external side. The lipid bilayer of the outer membrane is made of phospholipids in the inner leaflet of the bilayer and lipopolysaccharides (LPS) in its outer monolayer. Gram-positive cells lack the second outer membrane (300b).

The situation with Archaea is more complicated, likely reflecting the large diversity of extreme habitats to which Archaea have adapted (318). Archaea lack peptidoglycan, which was one of the features used originally to define prokaryotes. Two types of archaeal cells, which to date were found to harbor MG channels in their cell membranes, may illustrate this. The cell wall in halophilic archaea, such as *Haloferax volcanii*, whose cell membrane was the first to be examined for the presence of MG channels (239), consists of the S layer formed by a hexagonal arrangement of a glycoprotein (238). The protein appears to be anchored in the cytoplasmic membrane by a hydrophobic stretch found near the COOH terminus of the *H. volcanii* glycoprotein sequence (392). *Thermoplasma volcanii* is the second archaeon found to have MG channels in its cell membrane (220). This thermophilic archaeon has no cell wall, but instead contains an outer meshlike lattice of elements similar to nuclear lamins that is reminiscent of the CSK in animal cells (179). In addition, its cell membrane contains ether lipids based on 40-carbon, isopranoid-branched diglycerol tetraethers (361). In general, lipid bilayers of cell membranes of all archaea consist of diphytanylglycerol-diether or -tetraether or both (92). It is worth mentioning that neither bacteria nor archaea have a CSK in the eukaryotic sense. To describe the structural diversity of prokaryotic cell envelopes in its entirety would go over the scope of this review. What matters from the perspective of prokaryotic MG channels is that despite this diversity, it is always the lipid bilayer that is the tension-bearing element. The cell wall functions as a parallel viscoelastic structure that constrains the bilayer from excessive dila-

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**FIG. 2.** Cell envelope of Gram-positive (*A*) and Gram-negative bacteria (*B*). [From Volk (423).]
tion and in this way may reduce MG channel activation (41, 265, 267). The practical implication of the MG channel's sensitivity to bilayer tension is that, as discussed later, they retain their mechanosensitivity when reconstituted in liposomes (239, 388). The physiological implication is that they regulate cellular turgor by responding to bilayer stretch caused by osmotic swelling (2, 244; see sect. VI).

VI. MECHANICALLY GATED CHANNELS IN BACTERIA AND ARCHAEA

Since their initial discovery in giant spheroplasts of E. coli (267), the existence of MG channels in Gram-negative and Gram-positive bacteria has been amply documented (17, 18, 26, 27, 268, 387, 455, 456). The best-characterized are the MG channels of the Gram-negative bacterium E. coli, which have been studied by the patch-clamp technique in various giant spheroplasts (41, 76, 229, 266, 267) and in reconstituted membrane fractions fused with liposomes (18, 87). Based on their conductance and sensitivity to applied pressure, three types of mechanosensitive channels (Msc) can be distinguished: MscM (M for mini), MscS (S for small), and MscL (L for large) (17). The higher the conductance, the higher their activation by mechanical force transmitted via the lipid bilayer is that, as discussed later, they retain their mechanosensitivity when reconstituted in liposomes (239, 388). The physiological implication is that they regulate cellular turgor by responding to bilayer stretch caused by osmotic swelling (2, 244; see sect. VI).

A. Identification of the MscL Gene/Protein

The property of E. coli MG channels of being activated by mechanical force transmitted via the lipid bilayer (266) allowed for detergent solubilization and functional reconstitution of these channels into artificial liposomes amenable to patch clamp (388). Furthermore, it allowed application of a unique strategy of fractionation of E. coli membrane constituents by column chromatography and functional examination of the individual fractions for MG channel activity by patch clamp. This unusual approach led to the identification of a membrane protein underlying the activity of MscL (386, 388). The MscL protein was partially sequenced, which enabled the cloning of the corresponding mscL gene (384). The expression of the mscL gene alone in a heterologous and in an in vitro transcription/translation system demonstrated that the mscL gene alone was necessary and sufficient for MscL activity.

B. Structure of MscL

The mscL gene encodes a 15-kDa protein comprising 136 amino acid residues corresponding roughly to the 17-kDa protein band on a SDS-PAGE originally identified as the MscL protein (386, 388). Manipulation of the mscL gene by recombinant DNA techniques enabled production of the MscL protein on a preparative scale, which in addition to functional studies allowed also for higher order structural studies of MscL. Two methods have been used for simple purification of the MscL recombinant proteins. The first method employs the glutathione S-transferase (GST) protein fusion technique to express MscL attached to a cleavable GST domain (168), whereas the second method uses a 6xHis polyhistidine tag for purification of the recombinant MscL protein on a Ni²⁺-NTA column (27). Both methods yielded functional MG channels in patch-clamp experiments. The 6xHis-tagged MscL protein was used for secondary structure analysis by employing both transmission Fourier transform infra-red spectroscopy (FTIR) and circular dichroism (CD) (7). The MscL secondary structure includes two α-helical transmembrane domains (M1 and M2) connected by a periplasmic loop (Fig. 3, A and B). Thus MscL belongs to the family of structurally related ion channels with two membrane segments that include the epithelial sodium channel (ENaC), the inward-rectifier potassium channel (Kir), and the ATP-gated (P2X) cation channel (37, 302). Using the PhoA fusion technique, Blount et al. (27) could demonstrate that the NH₂ as well as the COOH terminus of MscL were located within the cytoplasm.

The higher order structure of MscL was first assessed in cross-linking studies. In one study, the multimeric structure of MscL was examined either by cross-linking the protein in situ and visualizing the cross-linked products by Western blotting using MscL antibodies against a COOH-terminal peptide or by cross-linking purified 6xHis-tagged MscL in which purified proteins were cross-linked. Both approaches indicated that MscL might form homohexameric channels (26, 27). In another study, various cross-linkers were applied directly to bacterial cells in which the MscL protein was radiolabeled by [35S]methionine (170) and left open the possibility that MscL forms multimers of higher order other than a hexamer. However, recent reevaluation of cross-linking experiments using various cross-linking reagents indicates that MscL is a homopentamer (389, 270a). Preparative scale production of milligram amounts of the MscL protein allowed employment of electron crystallography to study the structural assembly of MscL. The crystallographic analysis of two-dimensional MscL crystals at 1.5-nm resolution indicated that MscL forms homohexameric channels in lipid bilayers (353). However, the three-dimensional X-ray crystallographic study by Chang et al. (60) solved the oligomeric structure of the MscL homolog from Mycobacterium tuberculosis (Tb-MscL) to 0.35-nm resolution and indicated this homolog is organized as a homopentamer (Fig. 3C) (for discussion of homohexamer versus homopentamer, see sect. VI).
C. Conductive Properties of MscL

MscL forms nonselective ion channels of a very large conductance. The absence of any cation/anion selectivity or saturation in channels conductance up to 2 M KCl indicates MscL forms a large water-filled pore (75, 390). The reported values for MscL conductance range from 0.9 to 4.4 nS. Some of the differences may reflect the different
D. Is MscL a Hexamer or a Pentamer?

It is important to establish the multimeric organization of MscL because, as previously discussed for alamethicin and gramicidin, this feature can have important implications for gating mechanisms. As indicated above, homohexameric (Eco-MscL) and homopentameric (Tb-MscL) structures have been reported from crystallographic analysis (60, 353). In addition, several reports based on cross-linking experiments contributed to the MscL structural controversy by showing that MscL may form hexamers (27) as well as pentamers (389). Moreover, tandems of two MscL monomers expressed as a single dimer protein formed functional channels in giant E. coli spheroplasts, indicating that the functional channels can be made from an even number of monomers (27). On this last point, it would be interesting to obtain two- or three-dimensional crystals of channels made of the MscL protein dimers.

Is MscL a hexamer or a pentamer? Probably it is safe to say that MscL forms pentameric channels taking into account the resolution of 0.35 nm at which the Tb-MscL three-dimensional structure was obtained (60). Moreover, at the resolution of 1.5 nm for the two-dimensional Eco-MscL crystals, a pentameric MscL structure is just as likely to be judged as a hexamer (353). A resolution of at least 1.0 nm would be required to conclusively distinguish between hexameric and pentameric structures of the two-dimensional MscL crystals (J.-L. Rigaud and J.-J. Lacaperre, personal communication). Nonetheless, it is worthwhile pointing out several peculiar details of the Tb-MscL structure and the experimental conditions at which the three-dimensional structure was obtained. According to the Protein Data Base (PDB) summary report, the Matthews coefficient \(V_m\) of 5.98 for MscL is high compared with known structures of other proteins in the data base, indicating that the MscL structure is an outlier. The coefficient is usually in the range between 1.5 and 4.0 for tightly and loosely packed proteins, respectively. Also, the Ramachandran Z-score of \(-5.671\) for MscL appears very low and suggests a very unusual backbone conformation of Tb-MscL that is probably due to some local uncertainties in the backbone side chain conformation (A. Oakley, personal communication). In addition, the Tb-MscL crystallization experiments were performed at a very low pH between 3.6 and 3.8. This pH is below the \(pK_a\) value of 4.25 of the glutamic acid residue E104 in the COOH-terminal domain of Tb-MscL, which probably had to be protonated to stabilize the MscL pentameric structure. At higher pH one might expect the five E104 residues of the pentamer to become negatively charged causing destabilization of the multimeric structure, unless there is charge compensation by neighboring basic residues or cations present in the surrounding medium (305). Change in pH is known to modulate significantly the pressure sensitivity of MG channels (as discussed later) and also induce structural changes in proteins (42). Therefore, at pH \(\sim 7\) the MscL tertiary structure might differ from the reported pentameric one.

E. Origin of MscL Mechano-sensitivity

Bacterial and archaeal MG channels provide a clear demonstration that microbial MG channels can sense membrane tension directly. The tension develops in the lipid bilayer alone and directly gates these channels as described by the bilayer model. Since it was first proposed in relation to bacterial MG channels (261, 266), the bilayer model has become, along with the tethered model, one of the two mechanisms used to describe MG channel gating (see sect. VIII C). In the case of MscL, the validity of the bilayer model has been amply documented (28, 168, 384, 387, 388, 390). Interestingly, the well-studied stretch-activated cation (SA-CAT) channel endogenous to Xenopus oocytes also appears to be gated by bilayer tension (448).

As the amount of negative pressure (i.e., suction) applied to a patch pipette increases, the MscL channel open probability also increases (Fig. 4A). Activation of MscL by pressure can be described by a Boltzmann dis-
Distribution function for the channel open probability \( P_o \) (Fig. 4B)
\[
P_o/(1 - P_o) = \exp[\alpha(p - p_{1/2})]
\]
(3)
where \( p \) is the applied negative pressure, \( p_{1/2} \) is the suction at which the channel is open half the time, and \( \alpha \) is the slope of the plot \( \ln[P_o/(1 - P_o)] \). Because the Boltzmann function is very often used to characterize the mechanosensitivity of MG channels in the literature, we will discuss the meaning of the terms \( p_{1/2} \) and \( \alpha \), first in relation to MscL and then other MG channels. The following exercise should be useful to laboratories studying MG channels that do not routinely image membrane patch movements and calculate the membrane tension changes.

As shown previously (147, 374, 375, 390, 448), most MG channels respond to mechanical forces along the plane of the cell membrane (membrane tension), and not pressure perpendicular to it. According to the model of Howard et al. (190), the free energy \( \Delta G \) is a linear function of membrane tension \( t \)
\[
\Delta G = t \cdot \Delta A - \Delta G_o
\]
(4)
where \( \Delta G_o \) is the difference in free energy between the closed and open conformations of the channel in the absence of the externally applied membrane tension and \( \Delta A \) is the assumed difference in membrane area occupied by an open and closed channel at a given membrane tension, whereas \( t \Delta A \) is the work required to keep an MG channel open by external mechanical force at the open probability of \( 0 < P_o < 1 \). Consequently, in this model, the size of \( \Delta A \) is considered the sole parameter determining the mechanosensitivity of channel gating (i.e., the larger \( \Delta A \) the more sensitive the channel). Evidence indicates that for non-MG channels, like the ACh receptor channel (417) and specific voltage-gated \( K^+ \) channels (56, 130), the movement of transmembrane helices is quite small. In contrast, large movements of MscL are needed to account for the large pore formation and the steep tension-response relation of MscL (390).

Using the expression in Equation 4, the Boltzmann function can be rewritten as
\[
P_o/(1 - P_o) = \exp[(t \cdot \Delta A - \Delta G_o)/kT]
\]
(5)
In the case of MscL, which is activated by membrane tension near the lytic strength of the bilayer (i.e., maximal curvature), it has been demonstrated that in that activating pressure range and assuming an elastic membrane with a \( K_A = 10^2 \) mN/m (see Refs. 373, 390), the tension will be nearly proportional to the pressure (i.e., there will be little change in curvature). Using a version of Laplace’s law
\[
t - t_{1/2} = (p - p_{1/2})(r/2)
\]
(6)
where \( r \) is the radius of curvature of the liposome membrane patch under external negative pressure \( p \) applied to the patch pipette. Thus, under these conditions, \( p_{1/2} \) and \( t_{1/2} \), the characteristic negative pressure and membrane tension, respectively, at which the channel is open 50% of the time can be assumed to remain constant for a mem-
brane patch during an experiment. Because the free energy difference $\Delta G$ (Eq. 4) is equal to zero when the open probability $P_o = 0.5$ ($P = p_{1/2}$ and $t = t_{1/2}$), consequently $t_{1/2} = \Delta G_o/\Delta A$ and $p_{1/2} = 2\Delta G_0/r\Delta A$, whereas $\alpha = r\Delta A/2kT$. Thus it follows that at least in liposome or bleb experiments in which parallel CSK elements are not variables in supporting bilayer tension, the slope term will provide a direct estimate of molecular rearrangements of a MG channel, as long as the diameter and shape of patch pipettes remain nearly constant throughout the experiments. A convenient expression can be obtained by multiplying $p_{1/2}$ and $\alpha$

$$\Gamma_{MGC} = p_{1/2} \cdot \alpha = \Delta G_o/kT \quad \text{(7)}$$

showing that the product $\Gamma_{MGC}$ is independent of the patch geometry. $\Gamma_{MGC}$ provides a direct estimate of the energy difference $\Delta G_o$ between the closed and open state of an MS channel, and thus it presents the very characteristic of any type of MG channel reconstituted into a defined lipid membrane. For example, according to several reports, a negative pressure ($p_{1/2}$) ranging between $\sim 7.7$ and $10.3 \text{kN/m}^2$ (i.e., $\sim 58$ and $77 \text{ mmHg}$) is required to activate MscL 50% of the time in liposome patches (3, 141, 246a, 220b), whereas the sensitivity to pressure (1/$\alpha$) of the MscL channels was found to vary between 0.6 and 0.8 kN/m$^2$ (i.e., 4.5 and 6.0 mmHg) with $\alpha \sim 1.67$ and 1.25 (kN/m$^2$)$^{-1}$ (i.e., 0.22 and 0.17 mmHg$^{-1}$), respectively (3, 75, 168, 220b). It follows that the average $\Gamma_{MscL} \sim 14$, and consequently, the average energy required for opening MscL is $\Delta G_o \sim 14kT$. This value is in reasonable agreement with an independent estimate of $\sim 18.6kT$ obtained for $\Delta G_o$ from $P_o$-membrane tension curves for MscL (390). Clearly, the interpretation of Equation 7 becomes more complicated for cell membranes in which the associated cortical CSK (i.e., an extrinsic factor) can alter the tension seen by the bilayer and thereby change the shape of the Boltzmann independent of the intrinsic properties of the channel protein (see Ref. 447 and sect. vii).

F. Extrinsic and Intrinsic Factors That Affect MscL and Other MG Channels

The apparent sensitivity of MG channels to membrane tension may be experimentally altered by the following treatments.

1) Lysozyme, which disrupts the peptidoglycan of the bacterial cell wall, irreversibly increases the pressure sensitivity of MG channels in \textit{E. coli} giant spheroplasts (41, 268).

2) Cytochalasin, which disrupts actin microfilaments, increases the pressure sensitivity of MG channels in chick muscle and snail neurons (142, 370).

3) Mechanical decoupling of the CSK from the plasma membrane (i.e., blebbing) in \textit{Xenopus} oocytes decreases the MG channel mechanosensitivity (154, 160, 447).

4) Amphipatic (amphiphilic) compounds can increase mechanosensitivity of bacterial (266) and eukaryotic MG channels (323, 372).

5) Deletion, substitution, or single-site mutations can either increase or decrease the native MscL mechanosensitivity (25, 28, 170, 316, 317).

6) Changes in pH can affect MG channel sensitivity in both ways, with alkaline pH increasing (143; Martinac, unpublished data) and acidic pH decreasing the channel’s pressure sensitivity (28; Martinac, unpublished data).

These examples illustrate how the apparent mechanosensitivity of a channel may be altered by extrinsic, intrinsic, or possibly by a combination of mechanisms. Specifically, \textit{examples 1–4} may involve alteration in the way mechanical force is delivered to the channel (i.e., by alteration of CSK or bilayer properties) without altering the intrinsic properties of the channel protein (see also adaptation, sect. viiiE). \textit{Example 5} may involve changes in the protein’s intrinsic mechanosensitivity, and \textit{example 6} may result from changes in both extrinsic (bilayer) and intrinsic (protein) properties.

In the specific case of MscL reconstituted into liposomes, the following example illustrates how, for a MG channel activated by lipid bilayer tension, the measured changes in $p_{1/2}$ and $\alpha$ can provide an estimate of the channel’s intrinsic physical properties and identify the contribution of specific structural domains to the gating mechanism. Specifically, Ajouz et al. (3) studied the effects of various proteases on pressure-dependent gating of MscL in an attempt to identify molecular domains of MscL responsible for mechanosensitivity. They demonstrated that both parameters, $\alpha$ and $p_{1/2}$, were dramatically affected by protease treatment, with the slope term $\alpha$ significantly increased and $p_{1/2}$ decreased (i.e., mechanosensitivity increased). The quantitative changes in $p_{1/2}$ and $\alpha$ caused by protease treatment were such that $\Gamma_{MscL}$ (Eq. 7) ranged between 12 and 18 (i.e., $\Delta G_o \sim 12–18kT$) compared with $\sim 14$ before protease treatment. Because $\Gamma_{MscL}$ and $\Delta G_o$ were hardly affected by cutting the extramembranous domains, it was concluded that neither the cytoplasmic termini (i.e., the NH$_2$ and COOH termini) nor the S2-S3 periplasmic loop (see Fig. 3A) contribute critically to the mechanical gating of MscL. Instead, it was proposed that these extramembranous domains resist the movement of the transmembrane helices that underlie the critical event in mechanical gating of MscL. Note this result disagrees with the electromechanical model described below that proposes the NH$_2$ termini gate MscL (141).
G. Where Is the MscL Gate?

Based on a mutagenesis study in which Gly-22 (E. coli MscL) was changed to all other 19 amino acid residues, Yoshimura et al. (443) concluded that by analogy with Ala-20 of Tb-MscL, Gly-22 should surround the Eco-MscL gate.

Indeed, the Tb-MscL has been found to be extremely stiff when expressed in E. coli and examined by the patch clamp. Specifically, Moe et al. (283a) found that the channel required twice the membrane tension needed to gate the Eco-MscL. In addition, their study showed that amino acid substitutions at the neighboring residue V21 had severe effects on the channel mechanosensitivity, indicating that besides G22, V21 also participates in the energy barrier of MscL gating (283a). By using the program VOIDOO/FLOOD (222), Oakley et al. (305) estimated that in the closed conformation MscL could be filled with water molecules to a point 2.4 nm below the periplasmic surface of the channel. Also, the second cavity at the cytoplasmic face of the channel was found to be water accessible. Consequently, most of the inside of the closed MscL contains water except for a hydrophobic stretch of 0.8 nm that includes the Gly-22 residue and forms a water-tight occlusion. Thus the hydrophobic channel gate is quite thin when compared with the 3- to 3.5-nm-thick membrane bilayer (60). If we accept that in the open state of MscL, the hydrophobic gate becomes exposed to water, as originally proposed by Cruickshank et al. (75) and later reiterated by Yoshimura et al. (443), then can the value of 18.6kJ/m² be explained solely by this process. Taking into account that 17 ml/m² is necessary to transfer a hydrophobic protein from an organic solvent into an aqueous environment (66), it follows that 18.6kJ suffices to expose a hydrophobic area of 4.42 nm² to water (18.6kJ = 7.521 × 10⁻²⁰ J). This area is relatively small compared with the total membrane-associated area of MscL (~140 nm²) and roughly corresponds to a half surface of 5 α-helices, each having a diameter 2r = 0.68 nm (396), with a height l = 0.8 nm corresponding to the height of the hydrophobic gate (i.e., 2πrl ~5/2 = 4.27 nm²). Consequently, this result indicates that most, if not all, of the channel opening energy of 18.6kJ is used to expose a relatively small buried hydrophobic surface of the TM1 helices to the ionic environment of the bulk solution surrounding the channel. This may explain why Aţou et al. (3) never observed a permanently open MscL and why the proteases did not affect the gating mechanism of the channel.

H. Mutagenesis Studies

Several studies have used recombinant DNA techniques (25, 27, 28, 169, 283) or a genetic approach (316) to dissect the MscL molecule in an attempt to identify the essential functional domains responsible for MscL mechanosensitivity. Blount et al. (27) showed that a short deletion of 3 amino acids from the NH₂ terminal (14 residues long) and the larger deletion of 27 amino acids from the COOH-terminal region (36 residues long) had little or no effect on the channel properties. However, when 33 COOH-terminal residues were deleted that included a charged cluster RKKEEP of 6 more residues, the channel activity was abolished (387). Furthermore, these studies demonstrated that single residue substitutions in the first transmembrane domain TM1 of MscL, in particular, lysine K31 (28) and glycine G22 (316, 443), led to major changes in the MscL activities that could be correlated with phenotypes with major impairments in response to osmotic stress. In addition to its structural conservation throughout bacterial species, these studies emphasized the importance of TM1 in the MS properties of MscL.

Häse et al. (169) dissected MscL systematically by introducing deletions, additions, or large substitutions into the extramembranous domains. They reconstituted the mutated proteins into liposomes and examined their function. Consistent with previous findings (27, 387), large NH₂-terminal deletions or changes to the NH₂-terminal amino acid sequence affecting the overall charge of the NH₂ terminus were poorly tolerated and resulted in channels that exhibited altered pressure sensitivity and gating. Häse et al. (169) also confirmed the result of Blount et al. (28) showing that deletion of the charged cluster RKKEEP in the COOH-terminal domain abolished channel activity, possibly indicating MscL was no longer activatable by sublytic membrane tensions.

I. Models of MscL Mechanosensitivity

There is nothing in the three-dimensional structure of the closed conformation of Tb-MscL that unambiguously points toward a mechanism of how mechanical force gates the channel. However, several models based on structure-function relations are discussed below.

1. Multimerization model

One of the first models used to explain MscL gating was a tension-sensitive recruitment of MscL monomers into a pore-forming channel multimer (i.e., analogous to the alamethicin recruitment model in sect. IV A). This idea was based on pressure-response relations seen in many liposomes, as well as native membrane patches, in which channel activity (single-channel open probability and number of channels) continued to increase without saturation until the patch ruptured (168, but see Ref. 390). Furthermore, this “multimerization model” seemed to be supported by in situ cross-linking studies that indicated MscL existed predominantly as a monomer (of ~15 kDa).
in the \textit{E. coli} cell envelope (170) and the observation that functional channels could be formed from reconstituted MscL monomers electroluted from SDS gels (Saint and Martinac, unpublished data). However, in contradiction of the model, it was found that a much larger functional channel complex (\textsim 70–100 kDa) could be identified in gel filtration experiments (27) and that covalently linked tandem subunits produced the same size complex and MscL activity (27). We now know that the tandem result may occur because either the extra subunit does not participate in the functional pentameric channel or it is incorporated into a neighboring pentameric channel (e.g., see Fig. 7 in Ref. 389). Another result considered inconsistent with multimerization was the rapid opening transitions (i.e., \textsim 0.2 ms) of single MscL channels that were independent of MscL concentration and applied steady-state pressure (383). It was argued that fast transitions excluded diffusion-limited assembly of MscL monomers into functional multimers. However, it remains possible that there is an initial tension-triggered assembly of dispersed monomers into a multimer that is then gated cooperatively under steady pressure. To exclude this possibility, the latency for MscL opening in response to fast pressure steps (i.e., \textsim 1 ms) should be measured as a function of MscL concentration and step size.

2. Open-channel model

Based on a functional study in which large organic molecules were used to estimate the size of the MscL pore, an open-channel model of MscL was proposed that envisages 12 membrane-spanning \( \alpha \)-helices of a hexameric channel lining the pore of \textsim 4.0 nm diameter (75). Since the three-dimensional Tb-MscL structure indicates that MscL may be a pentamer rather than a hexamer (60), 10 transmembrane helices may line the pore of a smaller diameter channel that would have to be shorter to maintain the observed large conductance. The crystal structure of the closed MscL (Fig. 3B, Ref. 60) and the permeability properties of the open MscL indicate that MscL must undergo a large conformational change. For example, in order for MscL to open and let through molecules the size of thioredoxin (radius \textsim 1.8 nm and cytoplasmic side \textsim 0.2 nm of the pentameric channel \( \Delta A = \pi (r_{p}^{2} - r_{c}^{2}) \approx 10 \text{nm}^{2} \)). The same study calculated the free energy of transition from the closed to the open states to be 46.3 kJ/mol. This is comparable to the folding free energy of \textsim 54 kJ/mol required to completely unfold the 136 residues of the MscL monomer (\textsim 0.4 kJ \cdot \text{mol}^{-1} \cdot \text{residue}^{-1}) (305). Therefore, MscL must experience a radical structural change in channel opening that requires large molecular energies of the order expected during protein denaturation unless some unknown low-energy transition pathway exists between the two channel states.

3. Electromechanical coupling model

By recognizing the overall importance of charged residues, in particular in the NH\(_2\)- and COOH-terminal domain, Gu et al. (141) proposed an electromechanical coupling (EMC) model for gating MscL. In essence, the model proposes that the pore region of MscL is present in the closed channel and that the NH\(_2\) termini of the five subunits interact electrostatically with one another and pore regions to gate the channel (i.e., 5 swinging gates). Membrane tension by altering the tilt of the transmembrane helices is proposed to interfere with the balance of coulombic forces between the various domains of the channel molecule. The actual channel gating is accomplished by the flexible NH\(_2\) termini of each subunit pivoting around glycine G14 (i.e., located at the bilayer-water interface between the NH\(_2\) terminus and the TM1 transmembrane helix) to occlude the pore. Although the EMC model could account for some differences in mechanosensitivity of several MscL mutant channels (141), in its original form and with its underlying assumptions it suffers basic limitations. For example, one assumption was the fractional increase in pore area (\( \Delta A \)) and patch area during channel gating were the same (i.e., equal protein and patch elasticity) so that \( \Delta A = \) calculated (using Eq. 4) as only 0.78 \text{nm}^{2} with a free energy difference (\( \Delta G_{o} \)) of 2\( \text{kT} \). However, direct estimates \( \Delta A \) and \( \Delta G_{o} \) from \( P_{o} \)-tension curves give values of 6.5 \text{nm}^{2} and 18.6\( \text{kT} \), respectively (390). The model also did not take into account electrolyte screening of charged residues that would tend to negate any electrostatic interactions between the NH\(_{2}\) termini and the pore over the necessary long distances (i.e., \textsim 1 nm). Furthermore, the model predicts that MscL gating should be strongly dependent on ionic strength and membrane potential. However, no significant difference in MscL gating is observed in salt solutions ranging from 0.05 to 1 M or as a function of voltage (390). Furthermore, despite EMC model expectations, MscL function is not critically dependent on the length or charge of the NH\(_{2}\) terminal, since the removal of 3, substitution of 8, or addition of 20 new residues to this domain does not significantly alter MscL gating (26, 28, 169). Similarly, proteolytic cleavage of either the NH\(_{2}\) and/or COOH termini, rather than producing a permanently open channel as predicted, increases channel mechanosensitivity with-
out altering channel conductance (3). Finally, the three-dimensional crystal structure of the closed state of Tb-MscL indicates the channel gate involves a narrow pore region (∼0.2 nm in diameter) near the cytoplasmic ends of the TM1 domains (60). Despite these limitations, there are specific MscL structure-function relations that seemed to support the EMC model and have yet to be explained by other models. For example, the importance of charged residues in MscL gating has been well demonstrated by the deletion of the charged cluster RKKEE in the COOH-terminal domain that renders the Δ104 MscL mutant nonfunctional within the range of sublytic membrane tensions corresponding to pressures of 200 mmHg. The EMC model predicts the Δ104 mutant should only be functional at negative pressures of ∼300 mmHg or more (141). Also, the finding that very low pH (i.e., between 3.6 and 3.8) was a prerequisite for the successful three-dimensional crystallization of Tb-MscL (60) supports the importance of charged residues. In particular, the glutamic acid residue E104 with a pKₐ 4.25 may need to be protonated to stabilize the closed configuration of the MscL pentameric structure (305). One might expect that at a higher pH the electrostatic repulsion between the E104 negatively charged residues would destabilize the closed MscL structure, thus contributing in some way to the channel opening. Nevertheless, evidence now indicates that rather than being involved in directly gating MscL, the NH₂ termini may modulate the MscL mechanosensitivity, possibly by resisting the movement of the transmembrane helices during channel opening as well as interfering with ion permeation to produce multiconductance state behavior (3). Therefore, the EMC model as well as another recently proposed molecular model for gating transitions of MscL (387a) are most likely incorrect by assuming that the NH₂ termini function as the channel gate. However, the mobility of the NH₂ termini remains a valid assumption in both models. It may also turn out that the EMC-type model and its assumptions (i.e., small pore area change and a mechanoelectrical “triggered” swinging gate) will be applicable to eukaryotic MG channels.

4. Five-state kinetic model

Based on an analysis of multiple conducting states of the single MscL currents, a five-state linear kinetic model was proposed (390). The linear scheme of four conducting and one closed state C₁ ⇌ S₂ ⇌ S₃ ⇌ S₄ ⇌ O₅ with sequentially increasing substate conductances was found to be the simplest model to estimate the transition rate constants kᵢ, kᵢ,j = k₀exp(α/kT) and their dependence on mechanical force. In the expression for kᵢ, k₀ is the component independent of membrane tension, whereas α determines the tension sensitivity. According to the model, k₁₂ between the closed C₁ and the first subconducting state S₂ is the only tension-dependent rate constant limiting MscL opening. The large energy of 18.6kT (46.3 kJ/mol) calculated for ΔG₀ accounts for MscL being almost always shut in the resting membrane. (Energy difference of 18.6kT can be calculated using Eq. 7 by dividing the tension of 11.8 mN/m required for the MscL open probability of 50% with the maximal slope sensitivity of 0.63 mN·m⁻¹·e-fold⁻¹ obtained from the Boltzmann distribution, Ref. 390). However, the model is probably incorrect by describing the MscL gating in terms of only five conducting states. According to the molecular dynamics simulations of protein conformations, one would expect the channel to exist in many relatively stable conformations on a very short time scale on the order of microto nanoseconds that would be well below the resolution of patch-clamp experiments. Thus only relatively long-lasting conducting states (≥10⁻⁴ s) characterized by sufficiently large currents would be detected. This is in accordance with another study proposing that the minimal number of the MscL conducting states was seven with one closed, five subconducting, and a fully open channel state (247). The study determined that the number of subconducting states varied with the applied pipette voltage (Liu and Martinac, unpublished data), which is expected if the occupancy of different subconducting levels changes with voltage. This may explain why in the study by Sukharev et al. (390) fewer number of conducting states were detected, since the analysis was based on MscL currents recorded at a single membrane potential of +20 mV that was probably too low to resolve more than four conducting states. Nevertheless, the five-state model provides a correct estimate of energies involved in MscL gating by showing that the energy ΔG₀ necessary to open MscL is largely used to open the pore of 6 nm².

5. Hydrophobic surface match model

The hydrophobic surface match model derives from the original studies of the MS gating of gramicidin (see sect. nB) and represents an additional mechanism that may explain MscL. This model assumes that the hydrophobic match between the hydrocarbon bilayer thickness and the length of the hydrophobic surface of MscL is important in determining the stability of different channel conformations. The feasibility of the model will require information on the open MscL conformation so that estimates can be made of the free energy contributions of differences in hydrophobic mismatch between MscL states and the bilayer before and during stretch (i.e., bilayer thinning). Because it is already known that MscL gates close to lytic tensions, the bilayer must be near its maximal expansion and minimal thickness (i.e., 2–4% according to ΔA/A = −Δd/d; see sect. nB). A 2–4% change in bilayer with a thickness of 3.5 nm would thin the membrane ∼0.1 nm. If the thinned bilayer better matches the open channel than the closed channel by only 0.1 nm
(i.e., because it gets shorter as well as wider), this would produce an improved surface mismatch between MscL and bilayer of ~1.6 nm², assuming the diameter of MscL is 5 nm (60). Because the free energy increase for transferring a hydrophobic protein surface from an organic solvent to an aqueous environment is ~17 mJ/m² (66), the energy corresponding to the improved MscL/bilayer match should be \( \Delta G \sim 2.7 - 4.8 \times 10^{-20} \) J or \( \sim 7 - 12kT \). This energy is of the same order as the free energy difference of 18.6 \( kT \) estimated between closed and open states of MscL (390). Thus hydrophobic mismatch could presumably contribute to the mechanosensitivity of MscL. Indeed, it was recently found that when placed in a thinner bilayer MscL required less energy for activation by membrane tension; the opposite was true in a thicker bilayer (B. Martinac, unpublished observations).

In summary, the present experimental, structural, and theoretical evidence support the following simplified view of the MscL gating. The NH₂ terminus, although mobile, does not function as the channel gate. The gate is most likely formed by the hydrophobic constriction of the TM1 helices at the cytoplasmic end of the closed channel. The mobile NH₂ termini probably serve to stabilize the open channel conformation(s) and may interfere with the passage of ions, thus leading to channel subconductance states. The COOH termini possibly play a role in stabilizing the closed configuration of the channel, whereas the periplasmic loop may function as an elastic spring resisting the opening of the channel by membrane tension.

**J. Membrane Localization and Physiological Function of MscL**

MscL is a MG ion channel for which a physiological function can be correlated with its membrane localization and molecular properties. To begin with, two independent studies (27, 170) have associated MscL activity with the inner cytoplasmic membrane of _E. coli_ (i.e., the tension-bearing membrane). First, Blount et al. (27), using sucrose gradient centrifugation to separate total membrane of _E. coli_ into inner and outer membrane fractions, demonstrated the MscL protein was associated exclusively with the inner membrane fraction. Second, Häsé et al. (170) combined \(^{35}\text{S}\)methionine radiolabeling of MscL with the detection of the NADH oxidase activity in the cytoplasmic membrane fraction and showed 90% of \(^{35}\text{S}\)-MscL was associated with the NADH oxidase-rich membrane fraction. These results confirm the early study by Berrier et al. (18) that demonstrated MG channel activities were localized predominantly in the inner membrane fraction.

Bacteria exposed to an hyposmotic shock rapidly release cytoplasmic contents into the surrounding medium (38, 225, 344, 359). Moreover, osmotically induced efflux of lactose and ATP from _E. coli_ as well as ATP from _S. faecalis_ can be blocked by Gd\(^{3+}\) (19). Gd\(^{3+}\) blocks MG channels in _E. coli_ and other cells (158) and is proposed to act by modifying the mechanical properties of the bilayer rather than binding to the different channel proteins (105). Furthermore, two other types of MG channels found in _E. coli_, MscS and MscM, can also be activated by differences in osmotic pressure in patch-clamp experiments (77, 268). Although these results implicate MG channels in bacterial osmoregulation, what was lacking was a bacterial phenotype that would directly support such a role. Blount et al. (25) identified such a phenotype with several site-directed mutations (e.g., K31E or K31D in TM1) that led to dramatic changes in MG channel activities and also a “slow or no growth” phenotype that could be partially reversed by increasing osmolarity of the growth medium (25). Furthermore, these mutants showed a correlation between the severity of phenotype and the severity of “abnormalities” in ion channel activities.

A similar study by Ou and coworkers (316) found that when a randomly mutagenized _mscL_ gene was expressed in an _mscL−_ strain, which had no detectable phenotype on growth plates, most of the gain-of-function mutants, characterized by slow or no growth, could be associated with significant changes in the MscL channel kinetics and pressure sensitivities. Moreover, the most severe mutations occurred when amino acid residues between R13 and K31 in the first transmembrane TM1 segment of MscL were mutated. Significantly, the TM1 domain is highly conserved among bacterial species (283, 387).

Finally, Ajouz et al. (2) demonstrated that MscL opens in vivo during an osmotic downshock. Specifically, they investigated osmotically induced effluxes in wild-type and _mscL−_ mutant cells of small osmolytes such as potassium glutamate, trehalose, and glycine betaine that serve as osmoprotectants in bacteria. Although no difference was found between the wild-type and the _mscL−_ cells in the release of these osmoprotectants during osmotic challenge, thioredoxin, a small cellular protein, which is also excreted from _E. coli_ upon osmotic downshock, was completely released from the wild-type cells but retained by the _mscL−_ cells. This result indicates that MscL is not required for the excretion of osmoprotectants but is apparently activated in vivo to facilitate the efflux of thioredoxin. The effect of efflux of thioredoxin and possibly other small cytosolic proteins during osmotic downshock remains to be determined. Note the apparent excess MscL (i.e., 50–100 copies) expressed in single bacterial cells (26, 169), together with the fact that opening of single MscL channel would suffice to dissipate osmotic gradients within 1 ms, does not preclude their primary role in microbial osmoregulation or osmoprotection. This is simply because biological designs, particularly those underlying safeguard mechanisms, are not only based on economy but also redundancy.
K. MscS and MscM

MscS was the first MG channel activity described in bacteria (267) and was also the first MG channel shown to respond to bilayer tension (266). It has a conductance of \( \sim 1 \) nS in 200 mM KCl/40 MgCl\(_2\) (388) and requires \( \sim 0.7 \) the tension needed to open MscL (28, 387), but like MscL, it can also be activated by osmotic pressures (77, 268). MscS differs from MscL and MscM in that it exhibits a voltage dependence characterized by \( e \)-fold change in the open probability per \( \sim 15 \)-mV depolarization (267). MscS is relatively nonselective, displaying a slight preference for anions over cations (267, 388) and is blocked by submillimolar Gd\(^{3+}\) (17, 19). Recently, Booth and co-workers (31, 244) found that MscS activity in bacterial protoplasts is abolished by null mutations in two loci on \( E. \ coli \) chromosome, \( yggB \) and \( kefA \). The MG channels affected by the \( kefA \) and \( yggB \) null mutations have similar thresholds of activation by pressure, and both channels exhibit a conductance of \( \sim 1 \) nS. The activity of the \( yggB \) channel is encountered in almost 100% of protoplast patches and is characterized by a large number of channels gating simultaneously that inactivate rapidly with sustained pressure. The KefA activity is less frequently encountered (70% of the patches) and is characterized by fewer channels that do not inactivate. Whereas \( yggB \) is a small membrane protein of 286 amino acids, KefA is a large, multidomain 120-kDa membrane protein (1,120 amino acid residues). Interestingly, the amino acid sequence of \( yggB \) resembles highly the sequence of the last two domains of the KefA protein.

MscM is less frequently encountered in membrane patches of giant \( E. \ coli \) spheroplasts compared with MscS (76, 77) and MscL that are typically found in every patch (387). The conductance of MscM is about half that of MscS and exhibits a slight preference for cations (17, 18), indicating it is molecularly distinct from MscS and MscL. However, MscM is also blocked by Gd\(^{3+}\) and activated by hyposmotic stress (77). The different pressure sensitivities of the three channels (i.e., their activation pressures increase with their conductance) may indicate the channels are activated sequentially to provide a graduation of efflux pathways (124). However, apparently the activation of either MscS or MscL can maintain the integrity of \( E. \ coli \) during hyposmotic challenge, since single deletion mutants lacking either channel remain fully functional (31). In contrast, double mutants die, indicating that MscM alone is not able to protect bacteria from osmotic downshock.

L. MG Channels in Archaea

Archaea (formerly archaebacteria) are unicellular prokaryotes like eubacteria. However, they constitute a separate domain on the phylogenetic tree different from those of \( Bacteria \) and \( Eukarya \) (Fig. 5A) (318, 436). \( Archaea \) encompass several distinct groups of microorganisms adapted to extreme environments such as super-hot ocean hydrothermal vents characterized by extreme temperatures or Dead Sea containing extreme salt concentrations (13, 436). The existence of ion channels in archaean cell membranes has not been documented until the recent reports of porins (20) and MG channels (239) in the halophilic archaean \( H. \ volcanii \).

Two types of MG channels have been identified in \( H. \ volcanii \) (239) and have been named MscA1 (i.e., \( Archaeon1 \)) and MscA2. Both have large conductances and display a similar mechanosensitivity as the \( E. \ coli \) MG channels, but they differ in their distinct rectification properties. MscA1 has a conductance of 380 pS at +40 mV and of 680 pS at −40 mV, whereas MscA2 has a conductance of 850 pS at +40 mV and of 490 pS at −40 mV. Like the bacterial MG channels, both channels are activated by mechanical force transmitted via the lipid bilayer, and both are blocked by submillimolar Gd\(^{3+}\) (19).

With the use of the same functional approach as used to identify the MscL protein (384, 386), a 15-kDa membrane protein of the thermophilic archaean \( Thermoplasma \ volcanium \) was found to correspond to activities of a novel MG channel (220). In symmetric 200 mM KCl plus 40 mM MgCl\(_2\), the channel has a conductance of \( \sim 1.5 \) nS. Twenty NH\(_2\)-terminal amino acid residues of the 15 kDa were determined by microsequencing and were found to match with 75% identity the start of the open reading frame of a gene of unknown function in the genome of the related \( Thermoplasma \ acidophilum \) (Kloda and Martinac, unpublished data). Computer-assisted secondary structure analysis of the protein encoded by the \( T. \ acidophilum \) gene revealed two putative \( \alpha \)-helical membrane-spanning regions, suggesting a structural similarity with MscL. Helical wheel alignment of the two helices revealed that the first helix is amphipathic in character, has a cluster of five charged residues on one side of the wheel, and has a mixture of hydrophobic and hydrophilic residues on the other side and thus most likely lines the pore of the putative channel. This would be consistent with the three-dimensional crystal structure of Tb-MscL that indicates TM1 creates the bulk of the pore of the pentameric channel (60). The second helix has the usual \( \alpha \)-helix with hydrophobic and hydrophilic residues on both sides of the wheel.

Recently, a new MG channel was identified in the archaean \( Methanococcus \ jannashii \) by using the TM1 transmembrane domain of MscL as a genetic probe to search the microbial genomic database for MscL homologs (220a, 269). A hypothetical protein MJ0170 in the \( Methanococcus \) genome was found to contain a sequence that shares 38.5% identity with the TM1 of Eco-MscL. The same protein was also found to share a high homology
with the YggB protein underlying the activity of MscS in *E. coli*. This may indicate that MJ0170, which has been renamed to MscMJ for the MS channels of *M. jannashii*, is a hybrid of MscL and MscS, which may have evolved as a result of gene duplication of an ancestral *mscL*-like gene. Importantly, the alignment of sequences of MscL, MscS, and MscMJ homologs revealed that bacterial and archaeal channels form a phylogenetic tree composed of three main branches of prokaryotic MG channels (see Fig. 5B), indicating that the common ancestor of the prokaryotic MG channels most likely resembled MscL. When expressed in *E. coli* and examined in giant spheroplasts or after reconstitution into liposomes, the MJ0170 (MscMJ) protein expressed a channel with a conductance of $\sim 270$ pS in 200 mM KCl and a cation selectivity ($P_K/P_{Cl} \sim 6$) somewhat similar to eukaryotic SA-CAT channels (158, 265, 286).

**M. MG Channels in Evolution**

The finding of MG channels in organisms belonging to all three domains of the phylogenetic tree (Fig. 5A) points toward their early evolutionary origins. A basic question is whether the mechanisms of mechanical gating that originated in bacterial channels have been conserved in eukaryotic MG channels? Certain properties of the bacterial MG channels, including their extremely high
conductance, general lack of ion selectivity, and requirement of near-lytic activation tensions, have been taken as evidence that a different type of gating mechanism underlies the more MS and ion-selective eukaryotic MG channels. However, we now know from mutational and proteolytic cleavage studies that MscL can be made as MS (i.e., gated at or near zero applied pressures) as any eukaryotic MG channel studied in patch-clamp experiments. Furthermore, although a tethered mechanism (i.e., involving direct CSK- and/or EC-channel interactions) is often evoked for eukaryotic MG channels (see sect. VIII), there is growing evidence that at least some eukaryotic channels (e.g., the SAT-CAT channel in *Xenopus* oocytes) are gated by tension developed in the bilayer. As yet no sequence homologs for MscL have been identified in eukaryotes. However, there are striking similarities in the basic structure and membrane topology of MscL and the putative MG channels (MEC-4 and MEC-10) identified in *C. elegans* (403). This observation alone may indicate common biophysical principles confer mechanosensitivity on the two classes of channels. In the following section we discuss some of the additional cellular adaptations and specializations of animal cells (i.e., extrinsic factors) that play an important role in influencing how animal cells sense and respond to mechanical inputs.

**VII. THE STRUCTURE OF ANIMAL CELLS: SPECIFIC ROLES OF THE CYTOSKELETON AND EXTRACELLULAR MATRIX IN MECHANOSENSITIVITY**

One of the most important structures in terms of modifying the animal cell’s response to mechanical deformation is the cortical CSK (102, 108). This structure, by providing bilayer protection while preserving cell deformability, allows dramatic changes in cell shape and size during growth and differentiation and permits rapid animal movements. Furthermore, by removing the requirement of a thick cell wall, the cortical CSK allows the animal cell to maintain a more intimate contact with its mechanical surroundings. The basic function of the cortical CSK is to structurally support the fluid bilayer, thereby providing the cell membrane with a shear rigidity that is lacking in simple bilayer vesicles or spheroplasts/protoplasts. Furthermore, by acting as a membrane scaffolding, the CSK allows the cell to assume nonspherical geometries. As a consequence, animal cells are able to maintain a stable, excess membrane surface area beyond that required to enclose their volume as a smooth sphere. The biconcave RBC has 40% excess membrane area (107), whereas other cells (e.g., lymphocytes, skeletal muscle, mast cells, hepatocytes, astrocytes, and oocytes) may have between 100 and 1,000% in the form of membrane folds, microvilli, and/or cavaeolae (Fig. 8; Refs. 95a, 107, 338a, 375a, 444, 448). This allows the cell to increase in volume without new membrane insertion (312a, 338a, 375a, 448). Thus the additional surface area serves as an immediate membrane reserve providing compatibility between the highly expandable CSK network (\(K_A \approx 10^{-2}\) mN/m) and the nonexpandable lipid bilayer (\(K_A \approx 10^2\) mN/m) (107, 108, 284). During mechanical deformations (e.g., inflation, indentation, stretching), the CSK network may be expanded and the excess membrane smoothed out before significant tension develops in the bilayer (223, 330, 375a, 448). Several lines of evidence directly support this notion. First, scanning electron micrograph images of cultured mammalian cells indicate that osmotic swelling results in the surface microvilli “unfolding” as cells almost double in diameter (see Fig. 1 in Ref. 223). Second, mast cells can be inflated to nearly four times their volume with little increase in membrane capacitance (\(C_m\)) (i.e., <1%) (375a, see also Refs. 312a, 338a). Third, the force required to pull tethers (i.e., CSK-free membrane strands) from fibroblasts is independent of tether length up to ~5 \(\mu\)m (330). Fourth, direct inflation or osmotic swelling of oocytes to twice their normal diameter does not activate MG channels currents (448, 449). In contrast, only slight inflation (<10%) of CSK-deficient plasma membrane vesicles (PMVs) (i.e., formed from blebbled oocytes) activates MG channels (447).

Another effect the cortical CSK may have on bilayer mechanics is through organizing the bilayer into local domains with smaller radii of curvature \(r_c\) than that of the cell (an extreme case is the microvilli, \(r_c \approx 0.1\ \mu\)m, see below). According to Laplace’s law, this will effectively reduce the membrane tension \(t\) that develops in the local bilayer domain for a given osmotic or hydrostatic pressure \(p\) (i.e., \(t = p \cdot r_c^2\), see Ref. 145). Finally, the existence of contractile as well as load-bearing elements in the CSK allows the maintenance of resting cortical CSK tension that can actively resist membrane dilation caused by internally generated forces (15, 200).

The structure of the cortical CSK has been studied in greatest detail in the human RBC where it shows up in spread membranes as a hexagonal network of spectrin tetramers interconnected at their ends to actin, tropomyosin, and other proteins to form a multi-protein network (284). In resting RBCs, this hexagonal arrangement is obscured and appears in electron micrographs as a dense filamentous network, indicating the links in the network are normally folded (or bent) but can be extended with applied tension (144, 284, 377). For example, the distance between actin filaments in the resting RBC is ~70 nm but can reach 200 nm at full extension. Several molecular models have been proposed to underlie this large elastic extension, including random coil (377) and helical spring models (144, 274). The cortical CSK is anchored to the lipid bilayer by interactions between ankyrin and other CSK proteins with various integral membrane proteins.
(e.g., the band 3 anion exchanger). Although in nonerythroid cells there are many variations in both the specific cortical CSK elements (e.g., dystrophin in muscle) and the CSK-bilayer connections, the general organizational themes seen in the RBC appear to be conserved (250). The major difference between the anucleated human RBC and nucleated cells is that tension generated within the cortical CSK is not focused exclusively on the membrane but is also shared (or resisted) by the three-dimensional CSK network that suspends and transmits forces to the elastic nucleus. This CSK meshwork is highly integrated and has been proposed to behave as a tensegrity structure in which any mechanical deformation is transmitted globally throughout the network (200, 201). However, a recent study has demonstrated that fibroblasts show highly localized responses to mechanical deformation (171a). Also, mechanosensory neurons and receptors "sense" locally and transmit global responses electrically.

Although animal cells lack the rigid cell wall of bacteria, many animal cells (i.e., in tissues) possess a complex extracellular matrix (ECM) that serves as external scaffolding through which external forces can be filtered or focused on the cell or distributed throughout the tissue. For example, the physical coupling between ECM molecules (e.g., fibronectin) and the CSK through transmembrane proteins (e.g., integrins) allows forces to be focused on CSK elements that may directly or indirectly interact with membrane proteins (63, 327, 429). The viscoelastic elements in the ECM may also filter out steady-state forces while allowing the transmission of fast mechanical oscillations possibly through direct physical connections with membrane channels. Finally, specialized mechanosensory cells often possess unique structures (e.g., the tip links of stereocilia, Refs. 190, 326) and ancillary cellular layers (e.g., the Pacinian cell capsule) that confer high directional and vibrational sensitivity on the mechanotransduction process.

VIII. MECHANICALLY GATED CHANNELS IN ANIMAL CELLS

The original concept of MG channels arose from whole cell recordings of specialized mechanosensory neurons (89, 212, 249). In these early studies a physical mechanism of channel gating was often favored over an indirect chemical mechanism because of the typical short latency (i.e., a few ms) and high-frequency response (kHz) of the mechanotransduction process (70, 135, 242, 409). Tight-seal patch-clamp recording (153) allowed the direct measurement of single MG channels currents (37a, 142, 151), while pressure-clamp techniques (271–273, 347) and, in particular, the ability to apply fast pressure steps, demonstrated that MG channels in specific cell types (e.g., *Xenopus* oocytes) could be activated with millisecond latencies (271, see Fig. 6). However, not all cells show fast MG channel activation. For example, pressure steps applied to cell-attached patches on chick skeletal muscle (375) and snail neurons (370) activate MG channels with delays of 1–30 s. Furthermore, in snail neurons, physical or chemical disruption of the CSK abolishes the delayed activation. This indicates that the intact cortical CSK prevents or slows tension development in these cells (see sect. vii). This behavior illustrates how factors extrinsic to the channel protein can dramatically modify MG channel activity.

Patch-clamp studies over nearly 20 years indicate that MG channels are widely expressed in both sensory and nonsensory cells and in cells from species spanning the full evolutionary spectrum (see sect. viM). This ubiqui-
uity has led to the idea that MG channels play a role in general cellular functions such as cell volume regulation (151, 156, 231, 232, 265, 286, 348, 350, 358, 400). However, the possibility has also been raised that membrane changes induced by tight seal formation induce mechanosensitivity in specific channels. The “artifact” idea originally arose from a discrepancy observed between membrane patch and whole cell mechanosensitivity in snail neurons (287). Specifically, it was reported that despite the consistent ability to activate single MG K⁺ channels in membrane patches, macroscopic K⁺ currents could not be mechanically activated in the whole cell (287). Although this discrepancy has been shown not to be generalized to all cell types (55, 77, 84, 147, 192, 356, 362, 419, 433, 453, 454), it has remained a high profile controversy in the field. The issue has most recently been addressed in studies of Xenopus oocytes, where specific attention was focused on differences in the geometry and mechanics of the cell membrane in the patch and whole cell recording configurations (446–449).

A. Membrane Patch Mechanics and Morphology

The tightly sealed membrane patch spans the inside of the pipette with its circumference or boundary fixed to the walls of the pipette (153, 354). High-resolution video images indicate that in the absence of stresses normal to the plane of the membrane (i.e., due to hydrostatic or osmotic pressures) the patch appears to be pulled flat and perpendicular to the walls of the pipette (Fig. 7, Refs. 314, 373, 374, 448). This is consistent with Laplace’s law if

**FIG. 7.** High-resolution video images of a cell-attached oocyte membrane patch and membrane currents activated during brief steps of pressure and suction. A and B: video images of a membrane patch before (0 ms), during (50 ms), and after (250 ms) steps (i.e., of 100 ms duration) of suction (A) and pressure (B). C and D: the membrane patch currents recorded in the same patch imaged in A and B in response to the suction and pressure steps. The pipette tip (not visible) had a tip diameter of 4 μm, and the patch was located −20 μm from the tip. [From Zhang and Hamill (448).]
there is a resting tension in the patch. Presumably, the adhesive forces at the membrane-glass interface and, in particular, where the membrane is bent at right angles generates this resting tension (314, 373, 448). With applied suction (negative pressure) the patch flexes out (Fig. 7A) and with positive pressure flexes in (Fig. 7B). The patch appears optically smooth, and electrical membrane capacitance measurements indicate a patch area consistent with a flat membrane disk (448, see below). Furthermore, the expansion of the patch (i.e., <10%, Ref. 448) associated with rapid activation of MG channels (Fig. 7, C and D) indicates that little smoothing of the membrane is required before membrane tension develops. These observations are also consistent with electron micrograph images of oocyte patches in pipette tips that indicate a trilaminar structure with no evidence of membrane folds or microvilli (345).

In contrast to the simple membrane geometry of the patch, the plasma membrane of the oocyte and other animal cells displays a far more complex geometry. For example, transmission and scanning electron micrographs of *Xenopus* oocytes (Fig. 8) indicate extensive membrane folding and a high density of microvilli (29, 324, 444, 447). The quantitative analysis of freeze-fracture images of the oocyte indicate that membrane folding doubles the surface area while microvilli may further increase it by fivefold (444). This analysis is consistent with membrane capacitance measurements that indicate a membrane area that is 5–10 times larger than predicted for a smooth sphere (448). In the electron micrographic analysis, the estimated microvilli density was 6–7 microvilli/\(\mu m^2\) (with a microvillus length of 1.4 \(\mu m\) and a diameter of 0.12 \(\mu m\)). If the membrane folds and microvilli were preserved during tight seal formation, a patch with an apparent geometric area of \(50 \mu m^2\) (i.e., see Fig. 7) would have \(~300\) microvilli and an actual area between 250 and 500 \(\mu m^2\). However, \(C_m\) measurements indicate an area of \(~50 \mu m^2\) (448), again consistent with electron micrograph patch images that show no evidence of microvilli (345).

There are several scenarios by which tight-seal formation may alter membrane geometry (Fig. 8C). In one,

![Microvilli on the surface of *Xenopus* oocytes and their possible fates during tight seal formation. A: transmission electron microscopy of an oocyte showing prominent microvilli containing dark cytoplasmic material. B: scanning electron microscopy indicating the high density of microvilli on the oocyte surface. [Modified from Zhang et al. (447).] C: cartoon showing possible fates of microvilli during tight seal formation. Top panel shows a patch pipette pressed against the oocyte surface before application of suction. Middle left panel shows suction has drawn the membrane and tended to smooth out the microvilli both in and immediately outside the pipette. Middle right panel indicates suction shearing off (or excising) the microvilli in the patch. Bottom panel shows a tightly sealed flat patch after removal of suction.](http://physrev.physiology.org/)

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suction applied during sealing results in the lipid bilayer being decoupled from the underlying cortical CSK and dragged into the patch where it seals tightly to the pipette walls (281). This may be analogous to the drawing out of thin bilayer tethers from RBCs and other cells (181, 182). However, such tethers are much thinner in diameter than the membrane patch (i.e., ~0.2 vs. ~2 μm) and are completely free of CSK (182). In contrast, there is good evidence that a plug of CSK is drawn into the pipette along with the membrane (154, 345, 374, 447). Although, once the seal has formed the membrane cap can be mechanically decoupled from the underlying CSK to form a membrane bleb (154, 160, 374, 447). Thus a more likely scenario may involve the smoothing out of surface folds and microvilli (Fig. 8C) while retaining interactions with a reorganized CSK within the patch. In a third scenario, the microvilli are sheared off by the initial pressure/suction applied during sealing. In this case, one might expect the microvilli to be pinched off at their base with the membrane rapidly resealing or fusing. This may be analogous to the situation with an inside-out or outside-out patch that seals as the patch pipette is withdrawn from the cell (153). At least consistent with this idea is the observation that vesicles can be seen to be swept from the cell surface during tight-seal formation on chick skeletal muscle (374).

On the specific issue of membrane-CSK coupling, evidence indicates that both cell aspiration and tight-seal formation may alter the relationship between the bilayer and the cortical CSK. For example, in experiments in which RBCs were aspirated into pipettes without allowing tight-seal formation, fluorescent labeling of the CSK indicates a steep decrease in density of the actin and spectrin network along the aspirated membrane projection (91). Interestingly, this density gradient was maintained for the duration of the deformation (i.e., >30 min) but recovered rapidly (seconds) with release of suction. Similar changes may occur with “gentle” tight-seal formation that results in expansion, but not irreversible disruption, of the CSK network. As a consequence, the density of the network may decrease and membrane wrinkles and folds become smoothed out. However, stronger suction or pressure applied after the tight seal has formed may “overdilate” the network and thereby irreversibly disrupt links within the network as well as those between the network and the membrane (154, 374, 447). In this case, the membrane patch would lose its shear rigidity and elastic response to mechanical deformation. As a consequence it would behave more like a fluid flowing into the pipette to form a CSK-free bilayer bleb (e.g., see Fig. 10 in Ref. 447). Interestingly, Sokabe et al. (375) reported that suction steps caused slow progressive membrane patch movements with latencies of ~200 ms and a rise time of 1 s. These slow movements clearly contrast with the fast patch movements seen in Figure 7 and may be associated with decoupling of the membrane from the underlying CSK (see also Ref. 447). As a consequence of this decoupling, one might expect the ability to develop membrane tension in the patch would be reduced as more membrane flows into the patch (i.e., from along the sides of the pipette and the cell). Consistent with this idea is the reported decrease in mechanosensitivity (i.e., shift in the stimulus-response relations to higher pressures) in overstimulated patches and in blebbed membranes from Xenopus oocytes (375, 447, see Fig. 14A). However, as mentioned earlier, the mechanosensitivity of MG channels in snail neurons and chick skeletal muscle is increased after CSK disruption (370, 375). This may indicate that the CSK in these cells has a much higher resistance to expansion (i.e., >Kt) than the oocyte CSK and is more effective in preventing tension development in the bilayer. Such differences indicate that the viscoelastic properties of the CSK in each cell may be “tuned” to the mechanical requirements (functions) of the cell.

B. Discrepancy Between Membrane Patch and Whole Cell Mechanosensitivity

The above analysis provides some clues to the discrepancy between membrane patch and whole cell mechanosensitivity (370, 448). Specifically, the simple flat geometry of the oocyte membrane patch (i.e., before any mechanical decoupling) allows for rapid tension development and MG channel activation (Fig. 7). In comparison, the excess membrane area of the oocyte serves to buffer tension development and MG channel activation (Fig. 8). As mentioned previously, this explanation is also supported by the observation that while macroscopic MG currents cannot easily be activated in whole oocytes, they can be activated in oocyte PMVs (447). The PMVs lack a cortical CSK and take on a spherical geometry with no excess membrane area (i.e., as judged by electron microscopy) so that even slight inflation (1–2%) should increase bilayer tension. A similar explanation may account for why macroscopic currents can be activated in spheroplasts formed from E. coli, yeast, and other microbial cells (77, 146, 147, 454). To form spheroplasts, the parent cells are enzymatically treated to remove the cell wall and, as a consequence, the membrane blebs assume a simple spherical geometry so that even slight inflation causes MG channel activation (77, 146, 147, 454). Similarly, to evoke whole cell MS responses in smooth muscle cells, the cells apparently must be inflated to the point that they form whole cell membrane blebs or ghosts (see Fig. 1 in Ref. 362). One would also predict that in snail neurons, if the CSK can be disrupted (i.e., the cell blebbed) without causing cell rupture, then whole cell MG K+ currents would be activated similar to the single MG K+ channels in mechanically traumatized patches (see
Ref. 428). It remains to be determined whether the applied mechanical stimuli cause changes in membrane-CSK interactions in other cell types, such as vascular smooth muscle (84), urinary bladder myocytes (433), and cardiac myocytes (192), where whole cell MS currents have been reported.

Another cell type where membrane geometry may be important in determining mechanosensitivity is the kidney proximal tubule cell. In this highly polarized cell, two classes of MG channels have been recognized in membrane patches, a weakly cation-selective MG channel that is localized to the apical microvilliated surface and a $K^+$-selective MG channel that is localized on the relatively smooth proximal cell surface (see Ref. 351). Significantly, osmotic swelling or direct inflation of the cell can activate a whole $K^+$ conductance without activating the cation-selective conductance (55, 419). This differential sensitivity may reflect the location of the channels in regions of membrane with different geometry (i.e., different radii of curvature) such that they experience different tensions for the same applied pressure according to Laplace’s law (448). One can also imagine that other cells (e.g., mechanosensory neurons) possess localized regions of bilayer that are prestressed by CSK and/or ECM interactions to enable rapid tension increase in response to mechanical stress (i.e., analogous to the patch).

C. MG Channel Gating: “Tethered” Versus “Bilayer” Models

Bilayer reconstitution experiments have provided unequivocal evidence for a bilayer model of mechanical gating of alamethicin, gramicidin, and bacterial MG channels (see sects. iv and vi). However, it was apparently not intuitively obvious to early investigators (170a) that the fluid lipid bilayer with its low tolerance to dilation could develop and maintain tensions sufficient to influence membrane proteins. Indeed, in bilayer reconstitution experiments, phospholipid and cholesterol content are often adjusted to increase the expansion modulus of the bilayer and thereby shift the lytic tensions to higher values than those displayed by cell membranes (296). Furthermore, as discussed above, the excess membrane area of animal cells tends to buffer rapid increases in bilayer tension that otherwise might rupture the cell (79, 80, 330, 448). These apparent biases against the bilayer model would seem ample reason to consider an alternative model in which mechanical force is transmitted directly to the channel protein through CSK and/or ECM tethers. As discussed below, there are several lines of evidence that indicate a “tethered” mechanism may gate specific MG channels. However, as yet, there is no single experimental result (i.e., analogous to liposome reconstitution) that provides unequivocal support for this class of model.

1. Physical and chemical disruption of putative tethers

One strategy to identify a tethered mechanism is to show that disruption of the putative tethers abolishes MG channel activity. This strategy has been most successfully used in studies of audio-vestibular hair cells where extracellular “tip links” connecting stereocilia tips are hypothesized to act as external gating springs for MG channels (71, 326; Fig. 9). This model proposes that hair bundle displacement stretches the tip links and thereby influences the transition between the closed and open states.

![Diagram of a pair of stereocilia illustrating two different hypothesized sites of mechanotransduction. A: the tip-link between the stereocilia (a) is proposed to exert a gating tension on MG channels located at each attachment point (Pickles-Hudspeth model). Horizontal connections (exaggerated at b) between the stereocilia membranes where they come into closest contact are proposed to exert a shear displacement and gate MG channels located in the region of membrane abutment (Furness-Hackney model). B: force applied to the stereocilia (arrow) may cause distortion (stretch) of the membrane at a and b.](http://physrev.physiology.org/)
of the MG channel (69, 71, 73, 188–190, 263, 326, see Ref. 260 for recent review). Consistent with the model is the demonstration that tip-link disruption with external solutions in which Ca\(^{2+}\) is reduced by the presence of tetracarboxylate chelators (e.g., BAPTA) also abolishes mechanotransduction (10, 97, 263). Furthermore, after regeneration of the tip links (i.e., that takes several hours after disruption and chelator removal), mechanotransduction can be restored, although without the strong adaptation seen before disruption (451).

In the original version of the gating spring model, a two-state channel model was proposed (71, 188–190). However, this simple model (150) could not account for several features of hair cell mechanotransduction (see Ref. 260 for review). Therefore, a three-state model involving two closed states and a single open state (\(C_1 \leftrightarrow C_2 \leftrightarrow O\)) was suggested (73, 260). In the first closed state \(C_1\), it was assumed that a portion of its gating spring was immobilized so that part of the spring’s tension was not transmitted to the gate. As a consequence, this “latched” closed state would have a lower compliance than either the unlatched closed state \((C_2)\) or the open state \((O)\). It was assumed that the energy of channel opening depended linearly on hair bundle displacement such that the gating force was a constant independent of bundle position (69, 260). However, because the gating springs resist extension but not compression, they should slacken at negative displacement such that the channel’s total energy should become independent of negative displacement. This model could reproduce the observed asymmetrical displacement-response relation and account for the measured differences in bundle stiffness at negative and positive displacements as well as provide a good fit to experimental data from vestibular and cochlear hair cells (260). In this model, it was originally assumed that the gating springs were linearly elastic. However, recently it has been shown that their stiffness increases with tension, similar to other elastic materials (263).

Despite the necessary added assumptions, the tip-link/gating-spring model remains the main theoretical framework for hair cell studies. However, more recent observations have challenged the model (278). Specifically, it was reported that tip-link disruption by either BAPTA or elastase resulted in a sustained inward current that could be blocked by dihydrostreptomycin (100 \(\mu\)M), amiloride (300 \(\mu\)M), or Gd\(^{3+}\) (1 nM) (278). Because these three agents are known to block MG channels (although nonspecifically, see Ref. 158), it was proposed that the sustained inward current was carried through permanently open MG channels. This was considered inconsistent with the tip-link model because without the tip links, MG channels should be either closed or only show a low \(P_n\) consistent with their displacement independent energy evident at negative displacements (260). An alternative model referred to as the “abutment” model (Fig. 9) was proposed to explain the permanently open MG channels (119, 148, 278). This model originally arose from immunocytochemical localization of the putative MG channels near, but not at, the points of tip-link attachment (148). The putative MG channels were identified using a polyclonal antibody for the rat kidney amiloride-sensitive Na\(^{+}\) channel (rENaC) based on the observation that amiloride blocks both channels (158). However, amiloride blocks MG channels with a much lower affinity, and amiloride derivatives block with a different order of potency compared with the rENaC (343), raising questions regarding the specificity of the ENaC antibody for the MG channel. Furthermore, recent studies using α-ENaC knockout mice indicate that the α-subunit is not required for hair cell mechanotransduction (342). However, this may only indicate that other non-αENaC subunits make up the hair cell MG channel (see sect. viF2).

In the abutment model, the MG channels are located at the junctionlike structures where the stereocilia adhere. The resultant shearing forces at the membrane junctions during bundle displacement are proposed to cause channel opening, analogous to stretch-activated channels (see Refs. 142, 161). Recent kinematic analysis indicates that the putative channels at the abutment region could be operated as well as if they were located at the tip links (119). In particular, a linear change in shear displacement (i.e., at the contact region between the stereocilia) occurs as one deflects a stereocilia pair (within the physiological range of deflections), and these changes are comparable to the predicted tip link elongations (see Fig. 4 in Ref. 119). Furthermore, if the disruption of the tip links results in membrane junctions being exposed to increased tension, due for example to splaying of the stereocilia, then the abutment model might also explain why MG channels are permanently open (278).

It should be pointed out that others have reported even larger sustained inward currents following BAPTA treatment (10) [i.e., >400 pA (10) cf. with 40 pA (278)]. However, the currents were interpreted as arising from Ca\(^{2+}\)-dependent shifts in voltage-gated Ca\(^{2+}\) channel activation (i.e., to more negative potentials) and increased monovalent cation flux through the Ca\(^{2+}\) channels in the absence of external Ca\(^{2+}\). Significantly, the three agents used to implicate MG channels also block Ca\(^{2+}\) channels (158). On the other hand, there is no evidence that elastase could act this way on Ca\(^{2+}\) channels to produce the sustained current. Clearly, more selective agents will be required to unequivocally identify the nature of the sustained current and its relationship with MG channels. However, discrimination between the two models will probably only come with determination of the exact localization and nature of interactions between MG channels and specific stereocilia structures.

Another MG channel initially proposed to operate by a tethered mechanism is the endogenous SA-CAT channel.
in *Xenopus* oocytes (154). This idea arose because repetitive mechanical stimulation of oocyte patches irreversibly abolished rapid adaptation and reduced patch mechanosensitivity (154, see Fig. 14). On this basis it was proposed that critical CSK elements involved in gating and adaptation of MG channels were selectively decoupled from the MG channel. Consistent with this idea was the observation that during mechanical stimulation a clear space developed between the membrane and the underlying cytoplasmic structures (154, 447). However, subsequent studies found that MG channel activity was retained in membrane blebs and PMVs that lack any organized CSK based on immunocytochemical and electron microscopic evidence. This activity did not display adaptation, and the stimulus-response relation was shifted to higher pressures (447). On the basis of these new findings, it has been suggested that tension in the bilayer actually gates the oocyte MG channel but that the cortical CSK modulates the development and relaxation of bilayer tension (447). Interestingly, recent studies indicate that the CSK modulates the functional properties of non-MG channels (365, 399). Specifically, two independent groups have shown that mechanical-induced decoupling of the oocyte membrane patch from the underlying CSK (as described above) can alter the inactivation of voltage-gated Na\(^+\) channels by irreversibly switching the gating mode from a slow to a fast inactivation. The mechanism of this effect remains unknown. However, the Na\(^+\) channel is not stretch sensitive, since suction alone cannot activate the channel nor modify its voltage-dependent activation (cf., Refs. 365, 399).

Concerning attempts to biochemically disrupt CSK tethers, it has been demonstrated that cytochalasin not only does not block MG channel activation, it increases the mechanosensitivity of specific MG channels (142, 158, 370, 375). This indicates that actin microfilaments may normally constrain the development of tension in either the bilayer or other CSK proteins. Often spectrin (i.e., fodrin) has been proposed to act as a tether for MG channels (156, 348, 350). However, this idea has come from exclusion of other CSK proteins rather than any direct evidence, since there are no reagents that selectively disrupt spectrin. On the other hand, the recent demonstration that oocyte MG channel activity is retained in PMVs devoid of spectrin (i.e., based on electron micrograph images, Ref. 447) indicates that neither spectrin nor any other CSK protein is required to gate this channel.

The lack of effect of colchicine on MG channel activity in skeletal muscle (142) and *Xenopus* oocytes (158) and on tactile sensation in the cockroach (233) would also seem to rule out microtubules as gating tethers. However, colchicine treatment does abolish touch sensitivity in the nematode *C. elegans* and the cricket *Acheta domesticus* (59, 104). Furthermore, genetic and electron microscopic studies indicate that microtubules may be part of a complex that mediates touch sensitivity in nematodes and insects (161, 403, 409, 410).

2. Genetic disruption of putative tether proteins

Different genetic mutants provide evidence both for and against the involvement of specific EC and CSK proteins in MG channel gating. For example, the dystrophic (*mdx*) mouse, an animal model of Duchenne muscular dystrophy (DMD), is characterized by the absence of dystrophin, a large CSK protein normally expressed in vertebrate skeletal muscle. If dystrophin transmits force directly to the MG channels in skeletal muscle, then MG channels in *mdx* and DMD should be mechanically insensitive. However, stretch-inactivated Ca\(^{2+}\)-permeable channels are upregulated in *mdx* muscle (116), and stretch-activated Ca\(^{2+}\)-permeable channels can still be activated, although they may display abnormally slow closing (271). Therefore, although the absence of dystrophin may contribute to the elevation of \([\text{Ca}^{2+}]_0\) (i.e., via MG channels) that contribute to muscle degeneration (129, 416), the fact that MG channel activity is retained indicates dystrophin is not required for mechanical gating.

In studies of touch-insensitive mutants of *C. elegans*, Chalfie and colleagues have shown that mutations in the genes encoding a specific collagen in the mantle (i.e., mec-5; Ref. 95) and the \(\alpha\)- and \(\beta\)-microtubulin subunits in the CSK (mec-7 and mec-12; Refs. 195, 357) block touch sensation. This has led to the idea that proteins in the extracellular and cytoplasmic domains are tethered to the putative MG channels (i.e., MECs/DEGs, see sect. VIII F2). Interestingly, these studies indicate little redundancy in terms of transmitting mechanical force to the channel, since a single mutation in any one element is sufficient to abolish touch sensitivity. Although various models have been put forward for how these proteins may be organized (see Fig. 104, Refs. 120, 161, 403), direct evidence of MG channel activation (i.e., from patch-clamp studies) is still lacking. It remains possible that forces transmitted through ECM and CSK proteins do not directly gate the channel but instead stretch the bilayer. For example, early electron microscopic studies of the campaniform receptor of the fly by Thurm et al. (411) indicated a complex involving microtubules, membrane cones, and extracellular elements proposed to interact with MG channels analogous to the Chalfie model (Fig. 10B). However, it may be that the channels rather than being directly connected to the microtubules through the membrane cones are actually localized in the intervening bilayer regions that undergo increase in tension when the dendritic sheaf and membrane cones are compressed.

3. Identification of CSK protein binding sequences

The basic idea of the tethered model is that molecules in the CSK and/or ECM domains directly interact
with the MG channel protein. In this case, one would expect to find specific consensus regions or domains in the membrane channel protein that would allow such interactions. For example, COOH-terminal cysteine-rich regions (208) and NH2-terminal repetitive ankyrin repeats (425), identified in two recently cloned MG channel candidates, Mid1 and NompC (see sect. VIII), may mediate the protein-protein interactions of a tethered mechanism. On the other hand, they may serve to localize or cluster the MG channels in specialized regions of the cell rather than mechanically gate the channel. Certainly, there are other membrane transport proteins, including the RBC Cl− exchanger (band 3) and various ligand- and voltage-gated channels that interact with that CSK but are generally not thought to be mechanosensitive. Furthermore, a mutant form of the NMDA receptor channel in which the COOH terminus that normally links the channel to the CSK was deleted still displayed the same stretch sensitivity as the wild-type NMDA channels (53). This result, together with the observation that amphiphilic compounds modulate the NMDA channel’s stretch sensitivity, indicates a bilayer rather than a tethered model of gating.

4. Voltage sensitivity of patch mechanics and MG channels

Initial studies of membrane patch mechanics were interpreted as indicating that gating tension was developed in the CSK rather than the lipid bilayer (375). However, more recent studies indicate that the bilayer can
develop tension (4). In the initial study, prolonged pressure steps (i.e., ~5 s) were shown to increase membrane patch area beyond the expected elastic limit of the bilayer (i.e., >10%). This increase occurred with an initial delay of several 100 ms followed by a slow exponential (i.e., $\tau \sim 1$ s) rise during the pressure step and a slow exponential fall after the pressure step. These changes in area indicated $K_A$ values of ~50 mN/m, compared with values of ~500 mN/m for lipid bilayers (see sect. II). The lower $K_A$ values were interpreted as reflecting the more elastic (expandable) properties of the CSK network (107, 375). However, the authors also concluded that influx of new lipids from stores along the walls of the pipette could contribute to the large increase in patch area. The latter effect would also tend to lower estimates of $K_A$, but it was assumed that the bilayer alone could not produce an elastic recovery after the stimulus was removed (but see below). In the more recent study (4), the effects of membrane potential on patch breakdown were examined. In the case of lipid bilayers, it was already known that breakdown occurs when the sum of the energy due to tension-induced thinning and electrically induced compression exceeds a critical value (295). Therefore, it was argued that if the cell membrane breakdown was also voltage sensitive it would indicate the bilayer supports a significant fraction of the membrane tension (4). Indeed, patch-clamp results indicated that ~40% of the membrane tension was supported by the bilayer. To explain the reversible movements of the patch after the pressure steps (375), it was proposed that restoring forces were generated by the tendency of the membrane to be drawn back into the cell (4). This elastic recoil may be analogous to that displayed by thin lipid tethers that are pulled back by the lowered energy state associated with reestablishing interactions with the CSK (182).

Reports indicating membrane polarization causes membrane patch movement and activates MG channels are also consistent with bilayer tension gating the MG channel (127, 159, 177, 368, 449). However, although different groups agree that depolarization causes membrane patch movements, they disagree on the direction and reversibility of the movements and their contribution to MG channel activation. Specifically, Gil et al. (126) reported that depolarization (+50 mV) causes a slow (10–60 s) displacement of the membrane plug up the pipette (i.e., ~2 μm away from the cell) as would be expected with applied suction. Furthermore, they proposed that this patch movement caused the slow depolarization-induced activation of MG channels. However, it was not reported whether the plug could return to its original position to turn off the channels (see Figs. 1 and 2 in Ref. 126). In contrast, Zhang and Hamill (449) found that depolarization caused the patch to flex inward toward the cell (i.e., as occurs with applied pressure) without disrupting the patch boundary. This movement was reversible but did not consistently activate MG channels. Further studies on the role of patch geometry in the different patch pipettes may resolve the basis for the discrepancies. However, it does not appear to be due to difference in glass composition (126, 449). Both groups agree that the depolarization of the whole oocyte does not cause MG channel activation, presumably because the excess membrane area of the oocyte reduces the ability of electromechanical forces to create bilayer stress in the whole cell membrane (448).

5. Effects of lipophilic compounds

Recent studies indicate that the same lipophilic compounds that gate gramicidin (i.e., lysophospholipids and arachidonic acid; Ref. 251) and MsC (i.e., trinitrophenol and chlorpromazine; Ref. 266) in lipid bilayers also gate SA-CAT channels and MG K+ channels (i.e., TRED/TRAAC) as well as modulate NMDA-R channels (53, 257, 323, 372; see sect. III). This indicates that these eukaryotic MG channels may also be gated by bilayer tension similar to prokaryotic channels. However, because the compounds may act on the channel protein itself or on CSK proteins, liposome reconstitution of MG channel activity will be required to confirm a bilayer mechanism.

6. Reconstitution of purified proteins in lipid bilayers

The single criterion that can disprove the tethered model is demonstration that the purified MG channel protein retains mechanosensitivity when reconstituted into lipid bilayers. At this time, the only eukaryotic channel that this criterion has been applied is the ENaC (see sect. III). ENaC was reconstituted into planar lipid bilayers (PLBs) and a hydrostatic pressure gradient applied to stretch the bilayer (11, 202). This protocol was reported to reveal an unusual mechanosensitivity involving MS relief from Ca2+ block (203). In the past, the ability to develop tension in the PLB has been questioned because of the presence of a lipid torus (158, 350). Although it was argued the torus may be exhausted by large bilayer expansions (203), lipidosome patch-clamp studies of the ENaC (i.e., similar to MsC) should be carried out to confirm any tension sensitivity. More significantly, the reported mechanosensitivity seen in PLBs is not recapitulated in cell membranes (e.g., see Ref. 12). This leaves open the possibility that contaminant proteins form channels or that purification and reconstitution of the ENaC introduces novel properties due to incorrect folding and/or oligomerization. On the other hand, it may indicate that interactions with the CSK suppress the properties seen in the bilayer (51, 340). Studies of the ENaC in CSK-deficient PMVs from Xenopus oocytes (447) may discriminate between these possibilities. The PMV preparation should also be helpful in testing the feasibility of
reconstituting other MG channels heterologously expressed in the oocyte.

D. MG Channel Classification: Is There a Unifying Mechanism for Activation and Inactivation of MG Channels?

Like voltage- and receptor-gated channels, MG channels can be classified according to differences in their ion selectivity, conductance, and/or pharmacology. However, a more meaningful classification relates to their response to mechanical stimulation. For example, different MG channels vary in their sensitivity and response to pressure and suction applied to the membrane patch. A common classification is whether they are opened (i.e., stretch-activated channels; SAC) or closed (i.e., stretch-inactivated channels; SIC) by membrane stretch (143, 288). This classification refers to steady-state responses and ignores dynamic behaviors such as adaptation in which sustained mechanical stimulation may close SACs and open SICs (see below). Both SACs and SICs respond symmetrically to pressure and suction (i.e., PASA and PISI, respectively, see Fig. 11), indicating that it is membrane tension that actually activates or deactivates the channel. This symmetrical behavior may be mediated by tension developed in the bilayer or in CSK/EC elements that lie parallel to the plane of the bilayer. However, there are also MG channels that respond asymmetrically to pressure and suction and therefore may constitute additional classes. The first channel reported to display asymmetrical responses was a MG cation channel in rat astrocytes that was activated by pressure and inactivated by suction (PASI) (33–35). Subsequently, other groups reported similar behavior for MG cation channels in toad smooth muscle cells (178) and rat endothelial cells (191, 259). Recently, a possible fourth class of channel has been identified (PISA) in which suction activates a neuronal K⁺ channel but pressure has little effect (257, 323).

Figure 11 summarizes the four basic types of channel behavior in terms of idealized Boltzmann distributions. The asymmetrical responses to suction and pressure (Fig. 11, C and D) may arise with either a bilayer or tethered model of gating. For example, in the case of audio-vestibular hair cells, displacement of the stereocilia in opposite directions with respect to the graduated stereocilia axis either opens or closes the MG channels. This polarity-dependent behavior has been explained in terms of stretching or relaxing the extracellular gating spring (i.e.,

![Idealized Boltzmann distributions for different classes of channel behavior seen in response to suction and pressure applied to the membrane patch.](http://physrev.physiology.org/)

**A** PASA (SACs)

**B** PISI (SICs)

**C** PASI

**D** PISA

![Figure 11. Idealized Boltzmann distributions for different classes of channel behavior seen in response to suction and pressure applied to the membrane patch.](http://physrev.physiology.org/)

**A**: the stretch-activated channel (SAC) is symmetrically activated by pressure and suction (i.e., PASA), indicating it is responding to membrane tension. **B**: the stretch-inactivated channel (SIC) is symmetrically inactivated by suction and pressure (PISI). **C**: a channel that is activated by pressure but inactivated by suction (PASI). **D**: a channel inactivated by pressure and activated by suction (PISA).
tip links, see sect. vmiB2). Perhaps analogous CSK/EC structures that lie perpendicular to the plane of the bilayer, and are stretched in one direction and compressed in the other direction, are responsible for the asymmetrical responses to pressure and suction. However, asymmetrical effects may also arise in a bilayer model if pressure and suction cause differential expansion and compression of the inner and outer monolayer during patch movements. The following simple calculation indicates that this source of asymmetry may be significant. If suction deforms the patch from a flat circular disk to a hemisphere with a radius of curvature of 1 μm, then for a membrane thickness of 5 nm, the radii of curvature of the outer and inner monolayers will be 1 and 0.995 μm, respectively. This will result in monolayer areas of 6.28 and 6.22 μm², respectively (the reverse changes would be expected with a pressure-induced deformation). If the monolayers are coupled so that they cannot slide past one another and the number of lipid molecules in each monolayer remains fixed, then this would translate into proportional differences in both the area and the thickness of each monolayer. The difference, although only 1%, may be significant considering that the bilayer can rupture with each monolayer. The difference, although only 1%, may be significant considering that the bilayer can rupture with each monolayer. The difference, although only 1%, may be significant considering that the bilayer can rupture with each monolayer. The difference, although only 1%, may be significant considering that the bilayer can rupture with each monolayer. The difference, although only 1%, may be significant considering that the bilayer can rupture with each monolayer. 

Because mechanical gating arises from the channel protein being sensitive to some mechanical-induced deformation (i.e., either in the bilayer or in CSK/ECM elements), then adaptation could arise because of a relaxation in the force causing the deformation or a relaxation in the sensitivity to that deformation. Consider the simplest case of a two-state channel in which the rate constants for channel opening (β) and closing (α) are displacement sensitive (i.e., for a tethered MG channel) or tension sensitive (i.e., for bilayer-gated MG channel). The probability of the channel being open (P_o) will be given by

\[ P_o = 1/(1 + K) \quad (8) \]

where

\[ K = \beta/\alpha \quad (9) \]

or in terms of displacement

\[ K = K_0 e^{(x_0 - x)} \quad (10) \]

where \( K_0 \) is the equilibrium constant when the displacement \( x \) is equal to the set point \( x_0 \) and determines the number of channels open at zero relative displacement, and \( s \) is the sensitivity to the relative displacement change \((x_0 - x)\). For a bilayer-gated channel, we could substitute displacement with area change. An exponential time relaxation in either \( s \) or \( x_0 \) can produce the same adapting MG current (see Ref. 155). However, in one case there will be a reduction in sensitivity (i.e., a change in the slope of the response-displacement, \( P_o-x \), Boltzmann) while in the other case there will be a shift along the \( x \)-axis with no change in slope. [Note that in the simple and probably unrealistic two-state kinetic scheme (150, 260), the shift

E. Rapid Adaptation of MG Channel Activity

Adaptation to sustained stimulation is an important feature of many sensory receptors and is critical in allowing a receptor to ignore continuous or static stimuli and respond to transient or dynamic stimuli. Specific mechanoreceptor functions such as the pulsatile pressure sensitivity of arterial baroreceptors, the movement (acceleration) detection by vestibular hair cells, and the high vibration sensitivity of certain tactile receptors all depend on adaptation to maintain their high dynamic sensitivity over a broad stimulus domain. Although adaptation can arise at any stage of the transduction process, it is clear that single MG channel activity can display adaptation to sustained mechanical stimulation (35, 147, 154, 156, 168, 226, 285, 382). Figure 12 illustrates rapidly adapting MG channel currents recorded from an inside-out patch of a Xenopus oocyte (Fig. 12A), an isolated liposome patch in which MscL was reconstituted (Fig. 12B), and from a mouse utricle hair cell (Fig. 12C). The decay of the oocyte MG current is well approximated by an exponential with a time constant of ∼100 ms similar to the initial fast decay of the hair cell current but 10 times faster that the decay of MscL activity.

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and shape changes are clearly independent. However, in a three-state scheme, a change in set point may change the shape as well as produce a shift in the relation.[1] The latter mechanism is true adaptation because sensitivity is maintained, whereas the other mechanism is more akin to receptor desensitization or voltage-gated channel inactivation, where the stimulus must be removed for sensitivity to recover. For MG channel activity in the hair cell, the predominant effect of adaptation is a shift in the $P_{o-x}$ curve along the $x$-axis (8). Similarly, double steps of suction or pressure (Fig. 13) indicate that after oocyte MG channels have fully adapted they retain the same sensitivity to reactivation (154).

A number of factors have been shown to alter the rate of adaptation of MG channel activity in hair cells and oocytes. In both cell types, membrane depolarization causes a slowing of adaptation, and in the specific case of the oocyte MG channel, the slowing is a monotonic function of voltage with $-150$ mV depolarization causing an $e$-fold decrease in the rate of decay. However, unlike adaptation in audio-vestibular hair cells (9, 74, 98a), the oocyte adaptation and its voltage dependence is Ca$^{2+}$ independent (154). The oocyte adaptation mechanism may be located in the bilayer where it senses the electric field or alternatively coupled to a membrane protein that transmits electric field effects through a conformational change. Another notable difference between the hair cell and oocyte MG channel adaptation is the former’s directional sensitivity. For example, negative displacement of the hair bundle (i.e., toward the short-ended stereocilia) tends to turn off the channels, but then when the stimulus is removed, a rebound activation of channels occurs (Fig. 12C). This phenomenon presumably arises because adaptation results in the $P_{o-x}$ relation being transiently shifted to the left so that more channels will be open at zero displacement. In contrast, the oocyte MG channel shows no directional sensitivity to suction/pressure stimuli and no rebound channel reopening after the stimulus is removed. This difference most likely reflects the absence in the oocyte of the hair cell Ca$^{2+}$-sensitive myosin motor.

**FIG. 12.** Adaptation of MG channel activity in response to sustained stimulation measured in different membrane systems. A: inside-out patch recording from a *Xenopus* oocyte in response to a 10-mmHg suction step measured at a patch potential of $-130$ mV. The MG channel activity turned off with a time constant of 163 ms. B: MscL activity in a liposome patch in response to a suction step of 40 mmHg measured at a patch potential of $-10$ mV. The MscL activity turned off with a time constant of $-2$ s to leave some residual activity. [Modified from Hase et al. (168).] C: whole cell recording from a mouse utricular hair cell that was deflected ($-1$ nm) with a fluid jet under pressure-clamp control. There was an initial fast decay of current (i.e., over in $-50$ ms) followed by a sustained current. When the pressure pulse was turned off, there was a rebound outward current, indicating the MG channels at rest had been turned off. [Modified from Holt et al. (183a).]
that is proposed to actively reset the gating spring tension (see below).

For the hair cell, the voltage sensitivity of adaptation derives from voltage-dependent Ca\(^{2+}\) influx through the MG channel (9, 74, 189). However, within this framework, two distinctly different molecular mechanisms have been evoked to explain how Ca\(^{2+}\) might mediate adaptation. In one mechanism, internal Ca\(^{2+}\) interacts directly with the channel protein causing it to favor the initial closed state conformation C\(_1\) (i.e., in the kinetic scheme C\(_1\) ⇔ C\(_2\) ⇔ O, see Ref. 260), thus reducing the tension sensitivity because it would require more tension to put the channel in C\(_2\). As the channels close, Ca\(^{2+}\) sequestering processes lower internal Ca, and the C\(_2\) conformation is favored (73). This model has been recently revised to specifically account for the initial fast phase of adaptation (\(\tau \sim 1\) ms) that displays symmetrical kinetics (i.e., mirrored images) for small positive and negative displacements and is insensitive to myosin ATPase inhibitors such as vanadate (440). Furthermore, the fast kinetics of this phase contrast with the slower cycle time of a myosin ATPase motor that may contribute to the slow phase (\(\tau \sim 50\) ms) of adaptation.

In the second mechanism, the internal Ca\(^{2+}\) reduces the gating tension by causing a movement (i.e., by slippage of a myosin motor) of the tip link anchoring point to the MG channel, thus relaxing the tip link spring tension and thereby favoring the closed states. As MG channels close and internal Ca\(^{2+}\) falls, active motoring of the anchoring point retensions the gating spring. Consistent with this model, it has been shown that myosin ATPase inhibitors block the slower phase of adaptation (196, 440). It therefore seems possible that a combination of distinct mechanisms may contribute to adaptation. Overall evidence would seem to favor the second model in which the predominant effect of voltage, as with adaptation, is to shift the \(P_o\) curve along the \(x\)-axis rather than reduce its slope (i.e., sensitivity) (8). On the other hand, evidence indicates that Ca\(^{2+}\) may directly interact with the channel in a number of ways to either block the channel or induce channel conformational changes (see Ref. 155). Most recently, it has been demonstrated that external Ca\(^{2+}\) affects tip link elastic properties as well as their integrity (263). In contrast to MG channel adaptation in the oocyte, there is no intrinsic voltage sensitivity in either model of hair cell adaptation (i.e., it comes from voltage-dependent Ca\(^{2+}\) influx). However, both oocyte and hair cell MG channels show evidence of an intrinsic voltage-dependent conformational change that is proposed to underlie the voltage-dependent amiloride channel block (234, 343). The relationship between this voltage-dependent conformational change and other aspects of mechanotransduction has yet to be determined.

In oocyte patches, it is possible to irreversibly abolish adaptation of MG channel activity by either repetitive application of moderate stimuli (Fig. 14A) or by application of a single strong stimulus. This apparent fragility of adaptation (to sealing and stimulation protocols) explains why some groups have reported stationary kinetic behavior for the \textit{Xenopus} oocyte MG channel (154, 442). Interestingly, it illustrates an example where a single MG channel may be changed from a highly phasic receptor into a tonic receptor most likely by a change in the extrinsic properties of the membrane (see below). On the basis of the observation that during the loss of adaptation the membrane patch could be seen to be decoupled from the underlying cytoskeletal structures (154), it was suggested that viscous elements (dashpots) in the cytoskel-
eton become frozen or decoupled without disconnecting the gating springs (154, see also Ref. 35). Consistent with this idea was that MG channel activity recorded in CSK-deficient plasma membrane blebs or vesicles displayed little or no adaptation to sustained mechanical stimulation (447).

The above studies point to EC/CSK proteins tethered to the channel as being critical for adaptation of MG channel activity. However, recent studies of MscS in E. coli protoplasts (226) and MscL in liposomes (168) indicate that channel activities may display adaptation in the absence of CSK proteins (Fig. 12C). The adaptation of the E.coli channel activity is slower than that in Xenopus oocytes or hair cells (i.e., $\tau \sim 1$ s vs. 50–200 ms at similar voltages), is not voltage sensitive, and is not abolished by strong repetitive mechanical stimulation of the patch. Limited proteolysis applied to the cytoplasmic side of the patch reduces the number of MscS without removing adaptation, whereas stronger proteolysis abolishes mechanosensitivity (226). Note this proteolytic inhibition of MscS activity is opposite to the potentiation of MscL activity (3). Given that a bilayer rather than a tethered mechanism gate MscS, it was proposed that adaptation might be associated with insertion of the cytoplasmic domain of MscS in the bilayer (226). An alternative explanation is that the expansive force in the bilayer (i.e., due to bilayer bending, see sect. VIII D) relaxes as the monolayers slip past one another or lipids move from one monolayer to the other may (see Ref. 350). In this case, adaptation should be sensitive to the lipid make up of the monolayers and the degree of coupling between the two monolayers.

F. Structure of Eukaryotic MG Channels

Recent progress has been made in identifying several eukaryotic genes that encode MG or putative MG channels. However, so far none of the structurally identified channels has been characterized in the same detail as
MscL, and in some cases (e.g., MECs, NOMPC), patch-clamp measurements have yet to confirm single MG channel activity. Nevertheless, the preliminary findings indicate that a number of different molecular designs and mechanisms have evolved to confer mechanosensitivity on membrane ion channels. Below we discuss their key structural (i.e., topology, subunit stoichiometry, and protein-protein consensus domains) and mechanistic (i.e., bilayer vs. tethered) features.

1. A Ca\(^{2+}\)-permeable cation MG channel in yeast

Mid1 was originally identified from a mutant screen of yeast (Saccharomyces cerevisiae) induced to undergo Ca\(^{2+}\)-dependent mating-induced differentiation (199). Cloning and sequencing of mid1 indicated an integral membrane protein of 548 amino acids with at least 4 (and possibly 6) transmembrane domains (H1-H4) and 2 COOH-terminal cysteine-rich regions that may be involved in protein-protein interactions (i.e., that may localize and/or activate the channel) (208). Although Mid1 does not appear closely related to other membrane proteins, its H4 sequence shows similarities to the S3/H3 hydrophobic segment of voltage-gated Ca\(^{2+}\) and Na\(^{+}\) channels (208 and references therein). Heterologous expression of mid1 in Chinese hamster ovary cells was reported to increase resting Ca\(^{2+}\) membrane permeability, whereas stretch of the cell substrate (i.e., a silicon substrate) (i.e., a silicon substrate) displays a lower Ca\(^{2+}\) permeability (205, but see Refs. 207, 208a for corrections). Patch-clamp studies indicate that Mid1 is a SA-CAT channel with a high Ca\(^{2+}\) permeability \((P_{Ca}/P_K \approx 7)\). Although the Mid1 channel conductance \((32 \text{ pS in } 150 \text{ mM CsCl})\) is similar to that of a SAC previously characterized in S. cerevisiae protoplasts \((36 \text{ pS in } 170 \text{ mM CsCl})\), the protoplast channel displays a lower Ca\(^{2+}\) permeability \((P_{Ca}/P_K \approx 0.54)\) and significant anion permeability (147). Furthermore, recent results (X. L. Zhou, C. Palmer, and C. Kung, personal communication) indicate that protoplasts prepared from S. cerevisiae with the mid1 gene deleted still express the MG channel activity of the wild-type yeast protoplast (147). Although no homologs of Mid1 have been identified in higher eukaryotes, a potential Mid1 homolog, gam8, has been cloned from the fission yeast S. pombe and demonstrated to partially rescue S. cerevisiae from the mid1 mutant phenotype (402).

2. MECS: putative MG channels in C. elegans

Members of the Mec (mechanosensory abnormal) family in C. elegans were originally identified in mutant screens for touch-insensitive animals (57, 58, 59, 93, 122, 161, 403). The initial clue that specific mec genes may encode MG channels was that while recessive mutations in mec-4 resulted in touch insensitivity, dominant mutations in the same gene resulted in swelling-induced degeneration and lysis of the mechanosensory neurons (i.e., consistent with continuously open channels) (93). The cloning of mec-4 demonstrated that it was homologous to deg-1 that mediates swelling induced degeneration in other neurons (403). Together, Mec-4, Mec-10, and Deg-1 were proposed to belong to a protein superfamily called degenerins (DEGs). Other members of the MEC/DEG family in C. elegans include unc-8 (uncoordinated), expressed in motor neurons and required for normal locomotion; unc-105, expressed in muscle and required for stretch sensitivity; and flr-1 (fluoride resistance) required for normal defecation rhythm (248, 401, 404).

As yet, no MEC family member has been directly demonstrated to form MG channels. However, when unc-105 genes with gain-of-function mutations (i.e., predicted to cause constitutive channel activation) are expressed in Xenopus oocytes, they form spontaneous opening cation channels that display a variety of conductances (i.e., 2-30 pS) with no Ca\(^{2+}\) permeability (121). Amiloride blocks the Unc-105 channels, apparently by binding to a single site in the pore. This is similar to the amiloride block of ENaC (123) but different from the block of MG channels in Xenopus oocytes and hair cells where a voltage-dependent conformational change is proposed to expose multiple amiloride binding sites outside the membrane field (234, 343).

On the basis of sequence similarities, several other amiloride-sensitive Na\(^{+}\) channels have been classified as MEC/DEG family members. These include the ENaCs (50, 123), acid-sensing ion channels (ASIC) (424a), molluscan FMRFamide-gated channels, and Drosophila Na\(^{+}\) channels expressed in gonads [dGNaC1 or RPK (for ripped pocket) and in multiple dendritic neurons dmdNaC1 or PPK (for pickpocket) (1, 424a)]. The RPK and PPK were identified in Drosophila database searches that initially recognized a sequence homologous with a conserved region (i.e., in M2) in other MEC/DEG genes (1). All members of the MEC/DEG family are characterized by (1) two transmembrane domains (M1 and M2) with a single P-loop structure believed to line the channel pore, (2) intracellular NH\(_2\) and CCOH termini, and (3) a large extracellular loop (e.g., see Refs. 49, 403). In terms of secondary structure and membrane topology (but not primary amino acid sequence), the DEG/MEC family members are similar to MscL from prokaryotes (see sect. n.B) and also to the ATP-gated (P\(_{2X}\) receptor) channels (302).

Of all the DEG/MEC family members, the ENaC has been studied in greatest detail (50, 123). ENaC in most epithelia is composed of three homologous subunits: \(\alpha\), \(\beta\), and \(\gamma\), with each subunit proposed to contribute to the pore walls (357a). A fourth subunit, \(\delta\), mainly expressed in the testis and ovaries, shows properties similar to \(\alpha\) and may form heteromultimers with \(\beta\) and \(\gamma\) in these tissues (82a). Controversy exists regarding the subunit stoichiometry of the ENaCs, with some studies indicating \(\alpha\beta\gamma\)
(113) and others $\alpha_{\beta}\gamma_{3}$ (371a). Because the discrepancy may relate to different detergents used to solubilize and purify the ENaC protein, the results of a recent study that did not attempt to purify the channel complex are significant (105a). Instead, freeze-fracture electron microscopy was used to visualize the ENaC complexes expressed on the surface of *Xenopus* oocytes. The individual ENaC complex was seen as a square particle of $\sim 24$ nm$^2$, indicating $17 \pm 2$ transmembrane $\alpha$-helices (i.e., assuming 1.4 helix/nm$^2$) or 8 or 9 subunits (105a).

The fact that ENaC is a member of the MEC/DEG protein family has naturally led to the hypothesis that it is also a MG channel. Although some electrophysiological (11, 202, 203, 219) and immunocytochemical localization studies (94, 118, 148a) support this hypothesis, various methodological concerns and problems with interpretation have been raised (see Refs. 12, 158, 339, 350, 427). For example, the reported mechanosensitivity of ENaCs reconstituted into bilayers (11, 202, 203 but see sect. VIII) is not evident in the ENaCs expressed in cell membranes (12, 319). Furthermore, the report that heterologous expression of $\alpha$-ENaC in LM(TK) fibroblasts results in MG channels (219) appears problematic because that cell line expresses endogenous MG channels with similar properties to the presumed $\alpha$-ENaC (428). Finally, the results indicating that the $\alpha\beta\gamma$-ENaC subunits transfected in oocytes express a volume-sensitive inward current (cf., Refs. 11, 205) indicate, if anything, that the ENaC is a SIC, since cell shrinkage activates, while oocyte swelling inactivates the current (32, 288). The contribution of a recently reported endogenous shrinkage-activated current that is not mediated by the SA-CAT channel remains unclear (448, 449).

Despite the lack of compelling biophysical evidence for the ENaC being a MG channel, there are several immunocytochemical studies that have localized ENaC subunits in mechanosensory cells (94, 118, 148, 148a, but see Refs. 128, 342). One study reported expression of the $\beta$- and $\gamma$-ENaC subunits, but not the $\alpha$-subunit, in baroreceptor nerve terminals (94). Because $\beta$- and $\gamma$-subunits cannot form channels in the absence of the $\alpha$-subunit (50), another unidentified subunit must combine with the ENaC subunits to form the baroreceptor MG channel (94). The unidentified subunit may also confer Ca$^{2+}$ permeability on the channel (e.g., see Refs. 363, 391), since the $\alpha\beta\gamma$-ENaC is Na$^+$ selective (123). Interestingly, a more recent study has reported that $\alpha$, $\beta$, and $\gamma$-ENaC subunits are all localized in the perikarya of the trigeminal mechanosensory nerve terminals innervating the rat vibrissal follicle sinus complex (118). Furthermore, the subunits are colocalized with stomatin, a RBC protein homologous to *C. elegans* Mec-2 proposed to form the molecular link between the Mec4/Mec10 channel and the CSK (Fig. 10A, Refs. 195, 258). Significantly, the ENaC subunits and stomatin are not expressed in other specialized mechanoreceptors, although they are expressed in autonomic nerve cells (118). This may indicate that other MG channels (e.g., NOMPC, see below) or mechanisms are involved in mediating mechanotransduction in different mechanoreceptors or that the ENaC has another role in specific neurons not directly related to mechanotransduction.

### 3. NOMPC: putative MG channels in *Drosophila* and *C. elegans*

The gene *nompC* was identified in mechanoreceptive-defective (i.e., uncoordinated) *Drosophila* mutants that also showed an absence or reduction in the mechanoreceptor potentials (i.e., no mechanosensory potential) recorded from external sensory bristles (215, 425). *NompC* predicts a protein of 1,619 amino acids of which 1,150 NH$_2$-terminal residues consist of 29 ankyrin (ANK) repeats. ANK repeats have previously associated with forming complexes between membrane and CSK proteins (425) and may couple the channel to a CSK protein that gates and/or anchors (i.e., clusters) the channel in a subcellular region of the dendrite. The remaining 469 residues of NOMPC share some sequence identity (~20%) to the transient receptor potential (TRP) and TRP-like (TRPL) class of ion channels (67, 166a). The TRP channels also share structural similarities with vertebrate voltage-gated Ca$^{2+}$ and Na$^+$ channels, including six transmembrane domains (S1-S6) and a predicted pore region between S5 and S6. However, they lack the positive charged amino acids in the S4 region that confers voltage sensitivity on the channels.

Three *nompC* mutants with severely reduced MG currents had single nucleotide changes that introduced premature termination codons into their sequence. However, a fourth mutant (*nompC*') with normal-amplitude MG currents but accelerated adaptation involved a cysteine to tyrosine substitution at amino acid residue 1400 (i.e., between the S3 and S4 domains). This may be a critical residue underlying protein-protein or protein-lipid interactions that influence adaptation kinetics. Interestingly, screening of a *C. elegans* library revealed a homolog (Ce-NOMPC) that showed 40% identity with NOMPC and included the S1-S6 domains and the 29 ANK repeats. Furthermore, NOMPC and Ce-NOMPC were shown to be selectively expressed in ciliated mechanoreceptors (425, see also Ref. 209), whereas MEC/DEGs are expressed only in nonciliated touch cells (1, 403).

Heterologous expression and patch-clamp studies have yet to demonstrate that NOMPC actually forms a MG channel. Therefore, it remains unclear whether NOMPC can exist as a functional homomultimeric complex or whether it must interact with other protein subunits to form a MG channel (i.e., analogous to the MEC/ENaCs). The similar properties of mechanotransduction in *Drosophila* bristles and vertebrate hair cells (i.e., submilli-
second latencies, high directional sensitivity, and fast adaptation) may indicate a common tethered mechanism of gating (Figs. 9 and 10B, Ref. 411). However, liposome reconstitution or expression in CSK-deficient PMVs will ultimately be required to exclude a bilayer type mechanism.

4. SIC: a stretch-inactivated channel cloned from rat kidney

The capsaicin or vanilloid receptor (VR1) originally cloned from dorsal root ganglia (DRG) encodes a Ca\(^{2+}\)-permeable, nonselective cation channel and is a member of the TRP family (53a). In addition to being activated by capsaicin, it is also activated by heat, consistent with a role in nociception. Recently, a homolog of VR1 was cloned from rat kidney and shown to encode a large-conductance channel (250 pS) that was weakly cation selective and permeable to Ca\(^{2+}\) (393). Furthermore, the channel appeared to be a SIC because it was activated by cell shrinkage and inhibited by cell swelling (i.e., of sic transfected Chinese hamster ovary cells). The sic gene encodes a 563-amino acid protein with 6 transmembrane domains and a single pore region common to VR1. However, the NH\(_2\) terminal of the SIC is shorter than the VR1. Although it was reported that SIC was turned off by increased suction applied to the patch, it showed particularly slow recovery of activity (>1 min) after removal of the suction. The effect of increased pressure on channel activity was not reported (393). Other SICs in snail neurons (288), skeletal muscle (116), smooth muscle (178), and supraoptic neurons (311, 350a) display a significantly lower conductance (<50 pS), so it remains to be demonstrated whether they are structurally related to the rat kidney SIC.

5. TREK-1 and TRAAK: MG K\(^{+}\) channels in mammalian neurons

Recently, a new protein superfamily of weakly inward rectifying K\(^{+}\) channels has been identified that is characterized by four transmembrane domains and two pore-forming regions called P domains. The first family member identified was TWIK-1 (i.e., for tandem of P domains in a weakly ionic oocyte) (243). Subsequently, two other members, TREK-1 (related to TWIK-1) and TRAAK (opened by arachidonic acid), have been demonstrated to form MG K\(^{+}\) channels (257, 323). For example, when TREK-1 is expressed in oocytes, it shows a comparable sensitivity to suction as the endogenous channel but is less sensitive to pressure activation (323). Unlike the endogenous oocyte MG channel (448, 449), osmotic swelling and shrinkage increase and decrease, respectively, whole cell TREK currents. This may indicate the two channels are localized in different membrane regions (e.g., nonvilliated membrane vs. microvilli, respectively) so that they experience different tensions (i.e., due to the different radii of curvature, see Ref. 448). Alternatively, the two channels may involve different mechanisms of activation. For example, while the endogenous channel is most likely bilayer gated (448, 447), TREK-1 may be tethered. However, neither cytchalasin nor colchicine treatment was found to alter TREK activity (323, see below for TRAAK). Arachidonic acid and trinitrophenol (TNP) also open TREK-1. TNP is an ionic crenator of RBCs that activates bacterial MG channels. It may be that these amphipaths act on MG channels by altering the mechanics of the bilayer. However, direct effects on the channel proteins have yet to be excluded. It is also possible that stretch activation is mediated by stretch-induced release of arachidonic acid (e.g., via phospholipase activation). However, the lack of effect of delipidated bovine serum albumin, which should intercept fatty acid transmembrane (i.e., unless the release and receptor sites are extremely close), indicates stretch more likely acts by mechanical deformation of the bilayer. Kinetic measurements and the demonstration that mechanosensitivity is preserved in excised patches would reinforce this idea. Structure-activity studies of TREK-1 indicate that the COOH-terminal region is necessary but not sufficient for mechanosensitivity. Even more interesting, a charge cluster region resembling the COOH terminus of the MscL protein (RKKEE) appears crucial for the mechanosensitivity of both MG channels, indicating that a protein domain important in bacterial MG channel gating has been conserved in the eukaryotic channel (323).

TRAAD is similar to TREK-1 in that it can be activated by arachidonic acid, but it is only activated by suction in the cell-attached patch configuration and positive pressure in the outside-out configuration (257). This asymmetrical sensitivity indicates that a specific membrane curvature (i.e., convex) may be required for the channel to be activated (see sect. viID). Consistent with this idea is the observation that external but not internal application of TNP activates TRAAK. TNP is negatively charged and presumably acts by partitioning into and expanding the less negative external monolayer to induce a convex curvature. Unlike TREK-1 (323), it was reported that both colchicine and cytchalasin enhance TRAAK activity, indicating that these CSK elements may constrain tension development in the bilayer. As with TREK-1 (and MscL), there is a charged cluster region in the COOH terminus that is critical for both arachidonic acid activation and mechanosensitivity. Finally, although TRAAK is expressed in DRG neurons, indicating a possible role in mechanotransduction (257), it is also widely expressed in the brain, spinal cord, and retina, indicating a more general function in neuronal excitability (257a).

6. Orbiter, mercury, and gemini: mechanotransduction mutants in zebrafish

Specific genes encoding MG channels in specialized mechanoreceptors of vertebrates have yet to be identified. However, recent studies of zebrafish have identified...
a group of mutants that show defects in balance and swimming patterns (299). Of particular interest are three mutants, orbiter, mercury, and gemini, that have normal hair cell morphology (i.e., no signs of hair cell degeneration or hair bundle disorganization) and normal synaptic transmission but do not express a “microphonic” current when the hair bundle is mechanically deflected. Consequently, it was suggested that these genes may encode MG channels or ancillary proteins associated with transmitting force to the channels (299).

IX. MECHANOSENSITIVE ELEVATION OF INTRACELLULAR CALCIUM

Changes in [Ca\(^{2+}\)]\(_i\) regulate a wide variety of cellular processes, including cell growth and differentiation, cell motility and contraction, intercellular coupling, synaptic transmission, fertilization, apoptosis, and necrosis. It is therefore significant that mechanical stimulation triggers elevation in [Ca\(^{2+}\)]\(_i\) in many cells, including cardiac (366) and smooth muscle cells (218), fibroblasts (22), glia (62), osteoblasts (327), vascular endothelial cells (VECs) (88, 293, 294, 307, 364, 367), epithelial cells (30, 240, 435), hair cells (163), GH 3 cells (65), and smooth muscle cells (218), fibroblasts (22), and keratocytes (240). Apart from direct or gate the channels (299).

A. MS Ca\(^{2+}\) Influx Mechanisms

An early patch-clamp study of VECs identified a Ca\(^{2+}\)-permeable MG channel (235). Subsequently, MG Ca\(^{2+}\) channel activities have been proposed to mediate MS elevation of [Ca\(^{2+}\)]\(_i\) in heart cells (366), sensory neurons (134, 322, 363), hair cells (163), GH 3 cells (65), fibroblasts (22), and keratocytes (240). Apart from direct patch recording of channel activity, the other criteria used to evoke the MG Ca\(^{2+}\) channel mechanism has been the block of [Ca\(^{2+}\)]\(_i\) elevation by removal of external Ca\(^{2+}\) or by addition of Gd\(^{3+}\) (293, 294, 312, 366, 367, but see below). In addition to mediating direct Ca\(^{2+}\) influx, the MG channel may also indirectly activate voltage-gated Ca\(^{2+}\) channels by causing depolarization (65, 218). The Ca\(^{2+}\) entry through MG and voltage-gated Ca\(^{2+}\) channels may then trigger either Ca\(^{2+}\)-induced Ca\(^{2+}\) release (294) or activate phospholipase C activity and inositol 1,4,5-trisphosphate (IP\(_3\))-sensitive Ca\(^{2+}\) release (30, 39, 270), thereby further amplifying the increase in [Ca\(^{2+}\)]\(_i\). In turn, Ca\(^{2+}\) store emptying may produce a sustained Ca\(^{2+}\) influx through store depletion-activated (i.e., capacitative) Ca\(^{2+}\) channels in the plasma membrane that refills the empty stores (321).

B. MS Release of Ca\(^{2+}\) From Internal Ca\(^{2+}\) Stores

Mechanical stimulation, even in the complete absence of external Ca\(^{2+}\), can elevate [Ca\(^{2+}\)]\(_i\) by promoting Ca\(^{2+}\) release from internal stores (30, 62, 88, 197, 204, 300a, 307, 435). Two classes of mechanisms, indirect and direct, have been proposed to underlie this MS Ca\(^{2+}\) release. Examples of indirect mechanisms include the mechanical activation of either a MS phospholipase C (30, 39, 270) or a phospholipase A\(_2\) (206, 241, 307) that results in activation of the IP\(_3\)-sensitive Ca\(^{2+}\) release channel. Interestingly, addition of Ca\(^{2+}\) channel blockers (i.e., Gd\(^{3+}\), Ni\(^{2+}\), nifedipine, and nimodipine) in the absence of external Ca\(^{2+}\) causes a larger MS increase in [Ca\(^{2+}\)]\(_i\), presumably because they block Ca\(^{2+}\) influx through MG channels, voltage-gated Ca\(^{2+}\) channels (30), and/or store depletion-activated Ca\(^{2+}\) channels in the plasma membrane (307). Although it is possible that permeation of Gd\(^{3+}\) (and Ni\(^{2+}\)) through open MG channels could activate the fura 2 used to monitor [Ca\(^{2+}\)]\(_i\), and thereby produce an apparent rise in [Ca\(^{2+}\)]\(_i\), this mechanism could hardly explain the similar potentiation caused by dihydropyridines. Another type of indirect mechanism involves MS release of transmitter (e.g., ATP) and activation of receptor-gated Ca\(^{2+}\)-permeable channels (see sect. X). This mechanism has recently been shown to mediate the mechanically triggered spread of Ca\(^{2+}\) waves in prostate cancer cells (356a).

Evidence for a direct mechanical activation of Ca\(^{2+}\) release from internal stores has come from the response of VECs to osmotic swelling (204) and mechanical activation by twisting magnetic beads attached to the cell surface (300a). In the first case, Jena et al. (204) identified a novel internal Ca\(^{2+}\) store that can still release Ca\(^{2+}\) in response to hypotonic stress even after the plasma membrane’s permeability barrier had been selectively disrupted with the detergent saponin. Under these circumstances, mechanical forces are presumably transmitted to the Ca\(^{2+}\) channel in the endoplasmic reticulum (ER) by direct osmotic swelling of the ER. It was also demonstrated that under these conditions removal of external Ca\(^{2+}\) can rapidly deplete internal Ca\(^{2+}\) stores, and high concentrations of Gd\(^{3+}\) can block the internal Ca\(^{2+}\) release channel. These last results indicate the need for caution in using these criteria alone to implicate the Ca\(^{2+}\)
influx mechanism. In the second case, Niggl et al. (300a) demonstrated that VECs, astrocytes, and C6 glioma cells displayed a fast transient increase in [Ca\(^{2+}\)], in response to twisting magnetic beads attached to the plasma membrane (but see below). Because the Ca\(^{2+}\) increase also occurred in “sugar water” (i.e., ion-free solution), it did not involve Ca\(^{2+}\) influx. Instead, it was proposed that Ca\(^{2+}\) was released from IP\(_3\)-sensitive stores by different cell-specific mechanisms. For example, in VECs, the Ca\(^{2+}\) release appeared to be mediated by an MS increase in IP\(_3\) (based on inhibitor effects), whereas in C6 glioma cells it appeared to be mediated by direct mechanical activation of MG channels in the ER. The exact pathway by which forces are transmitted to the ER remains unknown. Internal Ca\(^{2+}\) release has been shown to depend on integrins (327), microtubules (406), and/or actin microfilaments (227). It has also been shown that the ER can be intimately associated with the plasma membrane (e.g., Ref. 122a). At least in C6 cells, neither cytochalasin D nor colchicine was found to block MS Ca\(^{2+}\) release (300a). A final question that arises concerns whether cells ever normally experience the mechanical stresses generated by twisting beads that attached to their cell surface. For example, if the beads undergo phagocytosis and/or cause reorganization of the surface membrane or CSK, then internal membrane Ca\(^{2+}\) stores may be made hypermechanosensitive, analogous to MG channels in tightly sealed membrane patches. For this reason, it is important to confirm that other forms of mechanical stimuli (e.g., fluid shear stress, cell stretch, or osmotic swelling) produce similar responses in the same cells.

### X. MECHANOSENSITIVE RELEASE OF TRANSMITTER

Although the major focus of research on mechanosensitivity over the last 15 years has been on the ubiquitous MG channel, it has recently become clear that MS release of transmitter, most notably ATP, is as equally ubiquitous in eukaryotic cells (45, 63, 139, 280, 292). Furthermore, in specific cell types such as the Xenopus oocyte, where both MG channels and MS ATP release are expressed, it is the MS ATP release that is more sensitive to mechanical stimuli (262, 292, 448). Perhaps most surprisingly is that MS ATP release has been implicated in vertebrate touch and stretch sensation (68, 292), two MS processes commonly assumed to be mediated by MG channels. Although MS ATP release has yet to be confirmed in mechanosensory neurons in situ, the question naturally arises how more than 50 years of research has failed to make the fundamental distinction between physical and chemical mechanisms of transduction.

### A. Historical Perspective

From a historical perspective, one can see how early studies of touch sensors may have been biased toward one mechanism or the other depending on the discipline of study. For example, the anatomists Spencer and Schaumburg studying the ultrastructure of Pacinian corpuscles (376) came to the conclusion that “a simple physical hypothesis of transduction does not take account of the elaborate array of organelles present at the base of each axon process. These organelles include numerous clear-core vesicles having the appearance and proportions of synaptic vesicles.” They further noted “that these clear-core vesicles in sensory axon terminals contain a substance which is released during the postulated mechanical distortion of an axon process and is then able to affect the ionic conductance of the axolemma, is an interesting speculation.” On the other hand, electrophysiologists in favoring a physical mechanism appeared to be more impressed by the speed and high-frequency response of specific tactile receptors. For example, Gottschaldt and Vahle-Hinz (135) studying Merkel cells concluded “the characteristics of the vibratory responses can hardly be explained in terms of chemosynaptic transmission. It is unlikely that a transmitter release mechanism could operate in phase with a vibratory stimulus of 1,200 Hz or more. Also, at such high frequencies an accumulation of released transmitter would be likely.” That the physical view has often prevailed would appear well justified in specific sensory systems such as hearing where the frequency response can be as high as 100 kHz (70, 196). Indeed, mechanically and ATP-activated currents in mouse outer hair cells have been shown to be independent and differentially blocked by α-tubocurarine (132). Nevertheless, the presumption that moderately fast kinetics (~10 kHz) must necessarily mean a physical rather than a chemical mechanism can no longer be justified by experimental evidence. For example, it has been demonstrated that transmitter release can be activated with latencies as short as ~150 μs (i.e., after the start of the action potential) and rise times in transmitter concentration can be as fast as 60 μs (40, 346). Furthermore, the idea that accumulation of transmitter may severely limit the frequency response needs to be revised in the light of rapid mechanisms for terminating the action of neurotransmitters, most notably ATP. Specifically, it has been demonstrated that nerve stimulation not only releases ATP but also soluble nucleotidases that would facilitate the ATP breakdown (413). Although the kinetics of transmitter release and breakdown are still not rapid enough to account for the >20-kHz frequency response of auditory transduction, they could be fast enough to mediate a tactile vibration sensitivity in the 1–2 kHz range (135). Below, we examine the kinetics and other features of...
mechanisms that may mediate chemical forms of mechanotransduction.

B. Tension-Sensitive Vesicle Recruitment/Exocytosis

Tension-sensitive vesicle fusion and recruitment can occur in artificial bilayer vesicles and specific cell types. For example, a flaccid bilayer vesicle will tend to lose smaller membrane vesicles by a process of budding and vesiculation until it becomes a sphere (i.e., the lowest energy state for a symmetrical structure under centripetal tension, Ref. 107). Similar membrane vesiculation/fragmentation also occurs in human and frog RBCs after disruption of their CSK (152, 284). Depending on a variety of factors that determine the spontaneous curvature of the bilayer (see sect. iiC), the parent vesicle (or RBC) may either eject vesicles into the external solution or inject them internally to produce an internal membrane reservoir. Increased membrane tension will tend to inhibit the budding (vesiculation) process by inhibiting the membrane invagination and neck formation between the parent membrane and the vesicle (78). On the other hand, increased pressure ensures the resting membrane is fully distended (i.e., no excess membrane) so that a rapid regulatory feedback loop exists between tension and surface area regulation. However, most animal cells maintain a stable membrane capacitance in response to osmotic swelling and shrinkage have been directly monitored by changes in membrane capacitance (2 fF) steps that may be used to exclude this form of artifact. Certainly, there are examples where osmotic swelling can activate a conductance increase without accompanying C_m increase (137, 338a), and vice versa (80, 129a, 427).

Tension-sensitive vesicle recruitment also results in exocytosis, since the vesicle will release its contents upon fusion with the plasma membrane. Stretching or inflating some cells has been reported to promote or facilitate exocytosis/release (63, 149, 262, 399, 352a, 435). However, the response is not universal (375a). For example, inflation of mast cells (i.e., to ~4 times their volume) actually causes a reversible block of exocytosis that recovers rapidly with deflation (375a). In this cell, exocytosis may require initial membrane dimpling to form the fusion pore with the mast cell granule, and tension presumably inhibits this dimpling (375a). Xenopus oocytes can also be inflated (e.g., to more that twice their without increasing C_m and/or activating MG channels (448). Both oocytes and mast cells display a large excess membrane area (i.e., 500%) that most likely protects their bilayer from tension changes except under the most extreme (pathological) conditions. In contrast, cells that respond to frequent changes in passive stretch and/or osmotic swelling (e.g., fibroblasts, urinary bladder epithelial cells, and mechanosensory neurons) may lack such a large excess membrane and thus rely on vesicle recruitment to accommodate stretch and cell volume changes (245b, 286a, 330). For example, during expansion of the urinary bladder, the epithelial cells undergo an initial smoothing out of surface folds on the urine-facing (i.e., apical) membrane followed by fusion of cytoplasmic vesicles with the apical membrane (245a). The membrane fusion/retrieval (i.e., as reflected in C_m changes) is reversible and occurs within 5 min (245b). In comparison, snail neurons show an increase (~10%) in C_m that takes between 0.5 and 3.0 min after exposure to a 50% hypotonic solution (80), and plant protoplasts undergo a pressure-induced increase in C_m that occurs with a latency of ~100 ms and a rise time of several minutes (457). In this case, membrane recruitment may be rate limited by random collisions between the vesicle and the membrane. In both cells, the decrease in C_m after return to isotonic (or hypertonic) solution (80) or release of pressure took 5–10 min. Although these kinetics may be adequate for preserving cell membrane integrity during relatively slow and sustained changes in membrane tension, they are too slow to enable responses to rapid cell deformations or movements and certainly could not transduce rapid oscillations in mechanical stimulation (i.e., necessary for tactile sensation). In contrast, stretch-facilitated transmitter release at synapses can dis-
play very fast kinetics (<1 ms, Ref. 63, see below), presumably because the vesicles are docked next to the cell membrane ready for rapid fusion. Another difference between synaptic exocytosis and tension-dependent vesicle recruitment is that the latter shows little or no Ca\(^{2+}\) dependence (see Refs. 174, 184).

C. Stretch-Facilitated Transmitter Release at the Vertebrate Motor Synapse

It has long been recognized that stretching skeletal muscle promotes transmitter release from the motor synapse (198, 415). This stretch facilitation is functionally significant because it can amplify the spinal stretch reflex. Most recently, Chen and Grinell (63) have shown that stretching frog muscle in the physiological range results in a 10% increase in release per 1% muscle stretch that is reflected in both increased frequency of miniature end-plate potentials (mepps) and amplitude of evoked end-plate potentials. The kinetics of the effect are extremely rapid, with the development and decay of the enhancement occurring in <1–2 ms. The stretch-induced enhancement of mepps is not dependent on Ca\(^{2+}\) influx and is not blocked by Gd\(^{3+}\) (63). At this stage, the enhancement mechanism remains unclear. However, the fast and symmetrical on-off kinetics as well as the low temperature sensitivity (i.e., Q\(_{10}\) – 1) seem most consistent with a direct physical mechanism, although biochemical changes occurring within a highly confined space cannot be excluded. The fact that the facilitation still occurs when incremental changes in intraterminal Ca\(^{2+}\) have been blocked (i.e., “clamped” at 100 nM) rules out stretch-induced Ca\(^{2+}\) release (63). Because integrin antibodies and binding peptides (arginine-glycine-aspartic acid, RGD) block the facilitation, mechanical forces may be transmitted intracellularly (via integrins) to alter the position or conformation of molecules controlling release (see Fig. 12 in Ref. 63). Although the RGD peptides block facilitation, they do not prevent stretch-induced elongation of the nerve terminal, indicating that other interactions between the muscle and the nerve remain intact but are incapable of transmitting stretch to the release mechanism. Interestingly, RGD peptides also block MS ATP release from Xenopus oocytes (262, see below). However, although ATP been shown to be coreleased with acetylcholine at the motor synapse (369) and a recent report indicates evoked ATP release from sensory neurons (381), evidence described below indicates that several mechanisms may contribute to MS ATP release.

D. Mechanosensitive ATP Release

Burnstock and colleagues (43–46) have advocated ATP's role as a neurotransmitter for over 30 years. More recently, ATP has been implicated in mechanosensation and cell volume regulation, by the demonstration that mechanical stimuli (including cell inflation, direct indentation, and osmotic swelling) can cause nonlytic ATP (and UTP) release from excitable and nonexcitable cells (139, 236, 237, 280, 292, 420, 430). The MS release of ATP (or UTP) may in turn induce an electrical or biochemical signal by activating purinergic receptors on the same cell (autocrine) and/or neighboring cells (paracrine). Two broad purinergic receptor families have been identified (303). One is the ionotropic receptor (P\(_{2X}\)) family, in which the ion channel is an integral part of the receptor protein (37, 64, 245). The other is the metabotropic receptor (P\(_{2Y}\)) family, in which the receptor is indirectly coupled to membrane ion channels either via soluble second messenger pathways or via membrane-delimited pathways (255, 289). Clearly, the class of receptor will influence the kinetics of channel activation and inactivation and thereby set limits on the frequency response of the transducer. This may vary from submilliseconds for the fastest ionotropic receptor channel to several minutes for metabotropic receptors (167, 176, 256, 264).

A key question for ATP release mechanisms is the nature of the driving force for ATP efflux. ATP in the extracellular milieu is normally kept extremely low (i.e., <1 \(\mu\)M) by extracellular ectonucleotidases (133). For example, Forrester (115) has measured basal levels of ATP of \(<2 \times 10^{-8} \) M in human venous plasma that may be increased ~50-fold (i.e., to ~1 \(\mu\)M) by partial arterial occlusion and exercise. Although ATP may be still higher in the interstitial fluid and even higher at the membrane surface near vesicular release sites, there should always be a steep gradient for ATP efflux, given that intracellular [ATP] is in the 1–5 mM range (360). The fact that ATP is also stored in cytoplasmic vesicles (424) indicates the existence of distinct pools of ATP that may be released by different mechanisms. For example, any stimulus that is strong enough to cause tissue damage will result in massive ATP release. It is presumably this mechanism that releases the ATP that mediates pain sensation. However, it is unlikely that ATP release caused by gentle mechanical stimulation arises from cell damage. For example, MS ATP release can occur without associated membrane conductance changes (i.e., in high impedance cells) and can be blocked by specific agents (262, 292, 448). Electroneutral ATP release can be monitored by the luciferin-luciferase luminescence assay or by the use of endogenous or heterologously expressed purinergic receptors as biosensors (139, 262, 292, 405, 430). A recent study using the luminescence assay has shown that collagenase treatment, RGD peptides, and cytochalasin can block MS ATP release from Xenopus oocytes. Furthermore, the ATP release saturates with repetitive mild mechanical stimuli (i.e., puffs of solution) at levels four orders of magnitude below those reached immediately after oocyte damage.
Electroneutral release of ATP may be mediated by an ATP electroneutral transporter and/or by vesicular release. Specific ATP transporters have been identified in the ER and the Golgi apparatus that translocate cytoplasmic ATP (176a, 328a). It is unknown whether these are functionally expressed in the plasma membrane. If ATP is released by exocytosis, one may be able to correlate it with changes in $C_m$ if there is a net increase in membrane area (see Ref. 129a). However, when exocytosis and endocytosis are exactly balanced, vesicular release can proceed with no detectable change in $C_m$. This is likely the case for the Xenopus oocyte that undergoes high rates of vesicle fusion (exocytosis) and fission (endocytosis) (i.e., 2,000–16,000 vesicles/s corresponding to 60–500 $\mu$m$^2$ membrane/s) capable of replacing the cell membrane every 24 h (444). This membrane turnover, which proceeds under basal (unstimulated) conditions, is associated with the delivery via vesicle trafficking from the Golgi apparatus and removal of membrane proteins. The vesicle trafficking between the Golgi apparatus and the plasma membrane can be blocked by brefeldin A (BFA) (118a). Interestingly, BFA also blocks basal and MS ATP release from Xenopus oocytes (262). Although membrane trafficking/ATP release does not require $Ca^{2+}$ influx, recent evidence indicates it may be increased by mechanical stimulation (262). The interesting implication here is that the protein and lipid composition of the surface membrane may be rapidly altered under specific mechanical environments. Because most eukaryotic cells share this vesicle trafficking pathway, it may account for the ubiquitous nature of ATP release from animal cells.

It has also been proposed that ATP permeates through specific membrane ion channels, most notably the cystic fibrosis transmembrane conductance regulator (CFTR) (52, 335) and the multidrug resistance (MDR) $Cl^-$ channels (4a). This idea remains somewhat controversial (360). However, the original argument that ATP was too large to permeate these $Cl^-$ channels appears invalid (246). On the other hand, there is no evidence to indicate that either CFTR or MDR are $MG$ channels. Another channel that might mediate ATP release is the hemi-gap-junctional channel (72). However, mechanical stimulation appears to close rather than open this class of channel (448, 450). Furthermore, it has been shown that submillimolar concentrations of $Gd^{3+}$ block the hemi-gap channel, yet even higher concentrations of $Gd^{3+}$ (1–10 mM) fail to block either basal or MS ATP release (262, Maroto and Hamill, unpublished data). Most recently, it has been reported that ATP is carried by a membrane conductance activated by strong hyperpolarizations of around $-200$ mV (29a). However, others have concluded that this conductance reflects reversible dielectric breakdown of the membrane, based on its lack of ion selectivity, failure to saturate, and slow (up to 10 min) recovery upon repolarization (449).

ATP release in the oocyte may be important in the cross talk with the surrounding follicular cells that express purinergic receptor-gated $Cl^-$ channels (324a). To account for the ubiquitous expression of ATP release from mammalian cells, it has been proposed that external ATP plays some role in establishing a set point for signal transduction pathways, in particular, those involved in changing $[Ca^{2+}]_i$, cAMP, and activating protein kinases (315). This set point may be important in influencing specific steps in cell development and differentiation. For example, ATP released from developing sensory neurons delays the terminal differentiation of surrounding Schwann cells and nerve myelination until they are exposed to axon-derived signals (381).

Nakamura and Strittmatter (292) have hypothesized that vertebrate touch sensation is mediated by an ATP-dependent mechanism of mechanotransduction. They propose that mechanical stimulation of the afferent nerve terminal causes ATP release that then results in autocrine activation of $P_{2Y_1}$ receptors. In support of this hypothesis, they demonstrated that touch-induced action potentials in frog sensory nerve were increased in frequency by subcutaneous injection of ATP, and this increase could be blocked by injection within the receptive field of a $P_2$ purinoceptor antagonist (suramin) or a ATP-degrading enzyme (apyrase). Subsequently, Svichar et al. (394, 395) demonstrated that ATP induces $Ca^{2+}$ release from $IP_3$-sensitive $Ca^{2+}$ stores in large DRG but not in small DRG neurons. Interestingly, they also found that ATP activated a large transient inward current that was over before the $[Ca^{2+}]_i$ began to rise, ruling out the transient current being activated by $IP_3$-sensitive $Ca^{2+}$ release. Another DRG study indicated a cation conductance activated by release of intracellular $Ca^{2+}$ stores (333). However, this conductance was also activated by heat and most likely mediates pain sensation. It remains unknown whether a related $Ca^{2+}$-sensitive conductance coupled to $P_{2Y_1}$ receptor activation mediates the generator (receptor) current in specific touch receptors.

Although the ATP hypothesis is attractive, several key elements of the hypothesis lack experimental support. To begin with, MS ATP release and its underlying mechanism need to be determined in the same large fiber DRG neurons that show the $P_{2Y}$ responses. Second, the relationship of this release process with $Ca^{2+}$-dependent exocytosis in DRGs (e.g., see Ref. 194) needs to be determined. For example, does the mechanosensitivity derive from $Ca^{2+}$ influx through MG cation channels? Third, the specific channel or conductance mechanism coupled to the $P_{2Y_1}$ receptor that presumably mediates the generator potential needs to be identified. Fourth, the underlying kinetics and sensitivity of both ATP release, channel activation, and ATP removal must be demonstrated to be...
compatible with the fast kinetics, high mechanosensitivity, and in some cases rapid adaptation of mechanotransduction in specific touch receptors. Fifth, it must be demonstrated that all the phenomena operate in the intact DRG nerve endings/specializations (see Ref. 379). Finally, the alternative hypothesis that tactile sensation is mediated directly by MG channels (e.g., MECs/DEGs and/or NOMPC homologs) needs to be rigorously examined at the functional level in different classes of tactile receptors. On the last issue, although it has recently been demonstrated that mechanical stimulation of the soma (273a, 400a) and growth cones (199a) of DRG neurons can induce conductance changes, they have yet to be related to specific single MG channel activities.

E. Membrane Resealing: $\text{Ca}^{2+}$-Induced Vesicle-Vesicle Fusion and Exocytosis

Apart from releasing cytoplasmic contents (e.g., ATP), a more fundamental response to membrane damage in terms of cell survival is the activation of mechanisms that repair or reseal the damaged membrane. Membrane repair/resealing may represent the most primitive form of cellular mechanotransduction. Studies in several different cell types have led to different models of membrane repair/resealing. One model, the “vesicle plug” model, has been used to explain the resealing of transected squid and crab axons (23, 100, 276), where it is proposed that $\text{Ca}^{2+}$-dependent formation and accumulation of endocytotic vesicles fuse to form a vesicular plug that reseals the transected axon. The resealing process requires high $[\text{Ca}^{2+}]_i$ (i.e., >100 $\mu$M) and calpain activation and occurs over a relatively slow time scale (i.e., minutes to hours) (23). A second model referred to as the “exocytotic” model has been used to explain resealing of micropunctures in sea urchin eggs and 3T3 fibroblasts (21). The resealing requires high $[\text{Ca}^{2+}]_i$, is completed within seconds, and has been correlated with bursts of exocytosis near the wound site (21). It is proposed that the vesicle fusion events supply the extra membrane needed to reseal the membrane wound. Unlike the first model, the vesicles are most likely present in the cytoplasm, possibly even docked close to the plasma membrane. Furthermore, because the resealing can be inhibited by neurotoxins that selectively interact with the SNARE complex proteins (e.g., synaptobrevin and syntaxin), the delivery, docking, and fusion of these vesicles with the membrane surrounding the wound site may involve a process similar to transmitter release (21, 382c). More recently, it has been reported that facilitation of resealing of 3T3 cells in response to a second disruption is blocked by BFA and cytochalasin, indicating that vesicle trafficking from the Golgi apparatus is involved (413a).

A third model, representing a composite of the plug and exocytosis models, has been used to explain the resealing of large disruptions of marine eggs and oocytes (407). Here, $\text{Ca}^{2+}$ elevation induces preexisting vesicles to fuse with one another to form a large single “wound vesicle” that then somehow fuses with the discontinuous bilayer (407). In both the exocytosis and composite models, the membrane repair may result in the release of signaling molecules (i.e., stored in the preformed vesicles) that mediate further responses, including cellular hyperplasia and/or hypertrophy that are commonly associated with mechanical stress (276). In the specific case of matured eggs, it is interesting that membrane damage (e.g., needle pricking) can promote the fertilization response, which involves $\text{Ca}^{2+}$-activated fusion of cortical granules with the plasma membrane (416a). Clearly, the secondary responses related to membrane repair will depend on the nature of the vesicles and their intracellular contents. For example, preliminary studies indicate that the yolk platelets, rather than cortical granules or endosomal vesicles, may mediate resealing of sea urchin eggs (407). The feature common to all models is that elevation of intracellular $\text{Ca}^{2+}$ triggers the resealing mechanism. It may be that sublytic membrane tension, by promoting $\text{Ca}^{2+}$ influx through SA-CAT channels, elevates local $[\text{Ca}^{2+}]_i$ and thereby primes the $\text{Ca}^{2+}$-sensitive repair mechanisms. In this case, the MG channel would act as a safety device somewhat analogous to MscL in E. coli. Patch-clamp recordings indicate the tensions that activate MG channels and cause patch rupture are within the same order of magnitude.

XI. CONCLUSIONS AND OUTSTANDING ISSUES

The response of both simple bilayer vesicles and cells to mechanical stimulation is determined by both extrinsic (e.g., size and surface area to volume) and intrinsic (i.e., material properties) properties. The deformation-sensitive membrane parameters that may likely influence membrane protein conformational changes include membrane dilation (i.e., increased area occupied by lipid molecules), the accompanying membrane thinning (i.e., assuming membrane incompressibility), and local changes in membrane curvature or bending. When a membrane protein is inserted in a bilayer it produces a mechanical deformation that depends on the coupling between the hydrophobic regions of the protein and the bilayer. A protein conformational change that involves a change in hydrophobic mismatch will be sensitive to both membrane thickness and local membrane curvature, each of which may be altered by changing either the lipid bilayer composition or by mechanically stretching or bending the membrane. The two simple channel-forming peptides, alamethicin and gramicidin, display MS channel gating that have been explained by two different classes of mechanisms. The
important distinction between the two is that whereas alamethicin channel formation may involve relatively large changes in membrane occupied area (i.e., associated with subunit recruitment into a barrel-staved complex), gramicidin channel formation involves insignificant area changes (i.e., a dimerization between monomers in each monolayer). As a consequence, the energy of gating of alamethicin but not gramicidin may reflect the product of the membrane tension (membrane dilation) and the occupied area change associated with channel opening. On the other hand, increased tension may act on gramicidin indirectly by causing membrane thinning, thereby affecting the free energy components that depend on bilayer-gramicidin hydrophobic coupling.

The purification, cloning, and recent determination of the crystal structure of the MscL protein from \textit{E. coli} and \textit{M. tuberculosis} have provided a rich environment for model building and testing. The present evidence favors a pentameric channel complex with the channel gate most likely formed by the hydrophobic constriction formed by the TM1 helices at the cytoplasmic end of the channel in the closed configuration. The large energy required to open the channel \((\sim 18 \text{ kT})\) may be needed to compensate for the exposure of the hydrophobic region to water that enters the open channel. The NH\textsubscript{2} terminus, although mobile, does not function as the channel gate but instead serves to stabilize the open conformation(s) of the channel and may interfere with the passage of ions, thus leading to the appearance of the channel subconducting levels. The COOH termini may function as an elastic spring resisting the opening of the channel by membrane tension. Clearly, future studies that provide structural information on the open conformation of MscL (e.g., by recently employed cysteine scanning mutagenesis combined with electron-paramagnetic resonance spectroscopy to probe the structure of MscL, Ref. 269a) will further refine this model.

Growing evidence in the form of amphipath activation and MG channel activity in CSK-deficient PMVs indicates that MG channels in specific animals cells (e.g., \textit{Xenopus} oocytes) are gated by increase in bilayer tension similar to prokaryotic MG channels. However, structural studies of several eukaryotic MG and putative MG channels (Table 1) indicate that a variety of molecular mechanisms may confer mechanosensitivity on membrane proteins. In some cases, a tethered mechanism of gating is favored (e.g., hair cell MG channels, MECS/DEGs, and NOMPC), but unequivocal evidence is lacking. Such evidence may come with the demonstration that 1) deletion of CSK binding sequences abolish mechanosensitivity, 2) rearrangement of CSK/membrane proteins (e.g., measured with fluorescence energy transfer) correlates with channel activation, and 3) mechanosensitivity can be restored in channel proteins reconstituted into liposomes by pulling on CSK/ECM labeled microspheres (i.e., that act as molecular handles).

In addition to the intrinsic properties of MG channel proteins, there are also various extrinsic mechanisms that modify the animal cell’s response to mechanical stimulation. For example, the excess membrane area of animal cells in the form of microvilli, membrane folds, and invaginations (i.e., caveolae) acts as a membrane reservoir to buffer sudden and/or large changes in bilayer tension.

<table>
<thead>
<tr>
<th>Channel Protein (Organism)</th>
<th>Conductance/Ion Selectivity</th>
<th>Amino Acids</th>
<th>Transmembrane Domains</th>
<th>Oligomerization</th>
<th>MS Gating Mechanism</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramicidin ((B. brevis))</td>
<td>15 pS/cation</td>
<td>15</td>
<td>1</td>
<td>2</td>
<td>Bilayer</td>
<td>6, 426</td>
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<tr>
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<td>1</td>
<td>≥3</td>
<td>Bilayer</td>
<td>47, 355</td>
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<tr>
<td>MscL ((E. coli))</td>
<td>−3 nS/nonselective</td>
<td>136</td>
<td>2</td>
<td>5</td>
<td>Bilayer</td>
<td>60, 387</td>
</tr>
<tr>
<td>MscS ((YggB) ((E. coli))</td>
<td>−1 nS/nonselective</td>
<td>296</td>
<td>3</td>
<td>Bilayer</td>
<td>31, 244</td>
<td></td>
</tr>
<tr>
<td>MscMJ ((M. jannashii))</td>
<td>270 pS/cation</td>
<td>350</td>
<td>3</td>
<td>Bilayer</td>
<td>220a, 269</td>
<td></td>
</tr>
<tr>
<td>MID1 ((\text{Yeast}))</td>
<td>35 pS/cation</td>
<td>548</td>
<td>4</td>
<td>199, 208</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRAAK ((\text{Human}))</td>
<td>30 pS ((0 \text{ mV})/\text{K}^+)</td>
<td>300</td>
<td>4/2P</td>
<td>257</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TREK-1/-2 ((\text{Mammalian neurons}))</td>
<td>48 pS/68 pS/\text{K}^+</td>
<td>538</td>
<td>4/2P</td>
<td>12a, 323</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIC ((\text{Rat kidney}))</td>
<td>250 pS/cation</td>
<td>563</td>
<td>6</td>
<td>393</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENaC* ((\text{Rat}))</td>
<td>10 pS/\text{Na}^+</td>
<td>638</td>
<td>2</td>
<td>5 or 9</td>
<td>Bilayer</td>
<td>50, 105a, 113, 203, 371a</td>
</tr>
<tr>
<td>MEC-4 ((C. elegans))</td>
<td>768</td>
<td>2</td>
<td>Tethered| 93, 403</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOMPC ((\text{Drosophila}))</td>
<td>1,619</td>
<td>6</td>
<td>Tethered| 215, 425</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGXO ((\text{Xenopus} \text{ oocyte}))</td>
<td>−80 pS/cation</td>
<td>Tethered| 277, 447</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGHC ((\text{Vertebrate hair cell}))</td>
<td>−100 pS/cation</td>
<td>Tethered| 74, 260</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* Mechanosensitivity has been reported for \(a\beta\gamma\)-epithelial \text{Na}^+ channel (ENaC) reconstituted in bilayers but not in cells. † Evidence for the mechanism is less compelling than liposome reconstitution. MG, mechanically gated.
that might otherwise rupture the cell (330, 448). As a consequence, bilayer-gated MG channels detected in tight-seal patch-clamp recording that tends to smooth out the membrane patch may not be exposed to activating tensions in the cell membrane except under special circumstances (447). For example, specialized mechanosensors may possess highly localized regions of bilayer that are prestressed (i.e., by CSK/ECM elements) to enable rapid response to mechanical deformation. Furthermore, cells that undergo changes in membrane geometry during growth and differentiation (96) or experience membrane reorganization or blebbing during specific cellular and physiological processes, including mitosis (328), apoptosis (216), cell locomotion (103), cell spreading (240), and organ distension (245b), may use up their membrane reserves and thus expose their bilayer to increased tension. However, some channels that display mechanosen-
sitivity in patch-clamp recordings [e.g., NMDA and S-type K+ (TREK/TRAAK) channels] may be of biophysical rather than physiological significance (287). Clearly, the identification of toxins that selectively block MG channels (e.g., the peptide toxin from Grammastola spatulata spider venom, Ref. 382b) and the development of genetic MG channel knock-outs may prove useful in demonstrating the function of specific MG channels (380, 434). It will be particularly interesting to use these tools to determine the possible relationship between MG channels and the MS processes controlling membrane turnover, vesicle trafficking, and membrane repair.

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Address for correspondence: O. P. Hamill, Physiology and Biophysics, UTMB, Galveston, TX 77555 (E-mail: ohamill@utmb.edu).

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