the substrate and the product of an enzymatic reaction—are present, then the ratio between the concentrations of those species will determine how many pores are blocked.

A test procedure using the described SSP-LUVs comprises the following steps. First, the substrate and the enzyme are incubated and a fraction of the substrate proportional to the enzyme activity in the sample is converted into product. Second, SSP-LUVs filled with a fluorescent dye are added. Because of the high concentration of the fluorescent dye, emitted light is reabsorbed and not emitted from the sample volume. The dye can only get from the inside of the LUVs (with its quenching conditions) to the outer medium (with its nonquenching conditions) via diffusion through the SSPs. The number of SSPs per LUV determines the maximal diffusion rate, which is diminished proportional to the concentration and affinity of pore-blocking molecules in the analyte.

Das *et al.* show in several examples that the substrate and product of enzymatic reactions often differ substantially in their pore affinity—the only property needed for their detection by the new system. The change in pore-blocking efficiency causes a related change in the dye's flux rate from the inside of the LUV to the outer medium, where it becomes detectable as a result of dilution. No laborious separation steps of bound and unbound fluorescent dye, as required in other techniques, are needed. The released fluorescent dye can be detected with simple, readily available equipment.

The system is attractive because of its simplicity and easy adaptability to new tasks. Das *et al.* show that the pores can be engineered to produce a variety of different affinities. In addition, several fluorescent dyes could be used if different LUVs are used in a single test. Because the principle behind this system—the differential measurement of the pore-blocking affinities of the substrate and product of an enzymatic reaction—is less dependent on the analyte structure itself than are antibodies, the SSP-LUV test system might be developed into a platform technology for high-throughput screening. It should enable sensitive fluorescence detection without any separation steps to remove bound from unbound dye.

Natural and synthetic pores form the basis of several exciting applications, demonstrating the enormous potential of such pores as nanometer-scale sensing elements. For example, the sequences of single-nucleotide chains have been determined during passage through an engineered nanopore (*2, 3*). Pores have also been used for ultrasensitive chemical detection (*4, 5*). Das *et al.* have added a new, promising application for engineered self-assembling pores.

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PERSPECTIVES: STRUCTURAL BIOLOGY

Force and Voltage Sensors in One Structure

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It is difficult being a prokaryote. Throughout their life cycle, bacteria and archaea may be exposed to a wide range of environmental challenges, including large changes in the osmotic activity of their environment. Prokaryotes have thus evolved several medium- to long-term membrane mechanisms that help maintain osmotic balance (*1*). In the face of sudden osmolality decreases, an emergency response is needed to prevent the cell membrane from bursting. In most prokaryotes this response comes from stretch-activated or mechanosensitive channels that act as the molecular equivalent of an escape valve to quickly balance any hydrostatic pressure buildup (*2, 3*).

There are two major families of nonselective mechanosensitive channels in bacteria: the large-conductance channel (MscL) and the small-conductance channel (MscS). On page 1582 of this issue, Bass *et al.* report the structure of MscS that not only gives new insights into tension dependent activation, but also provides a first look at a voltage-dependent channel (*4*).

MscL opens a pore with a conductance of about 3 nS, while MscS has a conductance of about 1 nS. In addition, MscS mechanosensitivity is significantly modulated by voltage (*5, 6*) so that its open probability (P_o) is increased by a factor of e per 15 mV of membrane potential change. Mechanosensitive channel activity can be reproduced in both MscL and MscS by reconstitution in lipid bilayers (*7*), suggesting that in these systems, the process of mechanotransduction is governed by lipid-protein interactions. Both channels were identified in 1987 with patch-clamp technology (*5*), but it took several years to determine their molecular structures (*8, 9*). MscS and MscL have no apparent sequence homology, but they act as part of a two-step mechanism of osmotic emergency response. MscS opens first, having an activation midpoint near 4 dyne/cm. MscL acts as a last-resort mechanism; its activation midpoint occurs at ~12 dyne/cm, close to the breakdown point of the lipid bilayer.

The first breakthrough in our molecular understanding of tension-dependent gating in mechanosensitive channels came with the determination of the MscL crystal structure (*10*). MscL (see the figure) is a highly α-helical homopentamer, formed by the association of short individual subunits (~130 amino acids in length) containing two transmembrane segments each. Now for an encore, the Rees group has done it again, reporting the crystal structure of MscS at 3.9 Å resolution (*4*). The channel forms a symmetric homoheptamer with a well-defined membrane region and a large, mostly β-containing extramembrane region that protrudes toward the cytoplasm. The membrane region has three helical segments, with the loop between transmembrane helix 2 (TM2) and TM3 and half of TM3 lining a central pore. The other half of TM3 is abruptly bent away from the pore and lies almost parallel to the plane of the membrane in its intracellular face. The pore is about 10 Å wide, consistent with the expected dimensions of an electrolyte-filled cavity having a conductance of about 1 nS, indicating that the structure corresponds to the open conformation of the channel. This is in contrast to MscL, whose crystal structure shows that it corresponds to the closed state. Because the tension needed to open MscS is about one-third of that needed to open MscL, it is possible that the crystallization conditions favored the open conformation of MscS. The structure is of a remarkably high quality even at 3.9 Å, as a conse-

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quence of sevenfold averaging and the high quality of the phases.

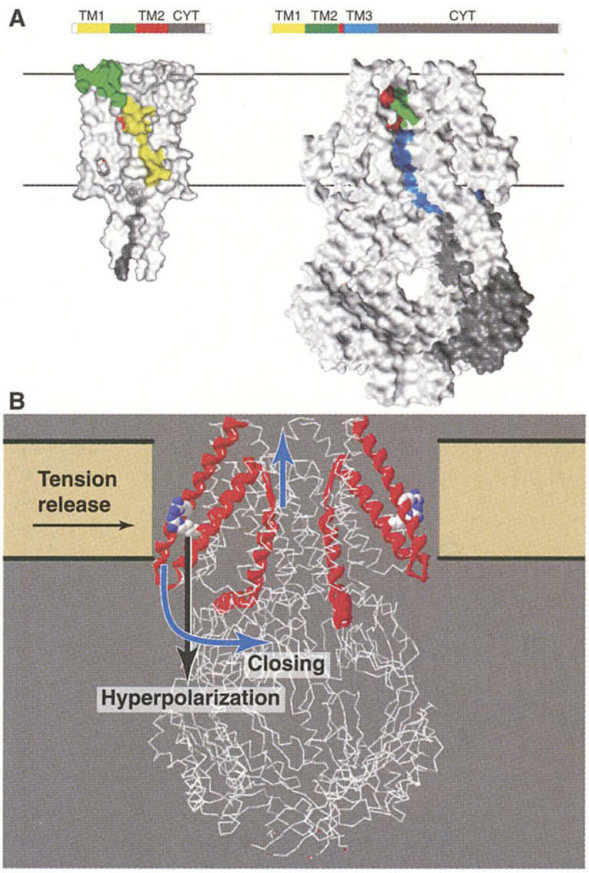
The mechanism underlying tension transduction of MscS is not known, but it likely differs from that suggested for MscL, given their significant structural differences. Thus, comparing these structures could give new insights into mechanisms of tension sensing. In addition, the new structure gives insights into voltage sensing, a mechanism that has not been elucidated in detail because no structures of voltage-dependent channels have been described.

In MscL, convincing evidence suggests that gating takes place through large helical tilts in both transmembrane segments, with the permeation path lined mostly by only one of the segments (*11, 12*) (see the figure). In MscS, the authors speculate that in the absence of tension, TM1 and TM2 would pivot on the extracellular side, making both helices more perpendicular to the membrane. This action would in turn carry TM3 toward the extracellular surface and straighten the break of TM3, presumably making the pore smaller. It is tempting to compare the gating mechanism of MscS with that of KcsA and the Ca^{2+}-gated potassium channel (*13*). The crystal structure of KcsA represents the closed pore (*14*), and it is made of four crossing helices that leave a minimal gap at the fourfold axis of symmetry. The Ca^{2+}-gated K channel, on the other hand, is thought to represent the open pore, and here a break at a glycine position in the inner helix allows for a wide opening. In a similar manner, TM3 of the MscS structure thought to be in the open conformation shows a break in position 113 (also a glycine).

If indeed the present structure represents the open conformation of MscS, it poses an interesting topological problem. Seven helices lining the pore impose a minimum distance of approach that leaves a gap of ~9 Å. This raises the question of how it is possible to close the conducting pathway with that number of helices and still maintain symmetry. To overcome the steric constraints of seven helices in close proximity, the loop connecting TM2 and TM3 could become part of the gate. Alternatively, the perfect symmetry shown in the open conformation might be broken in the closed state, allowing three or four helices to cross at one level while the others do it at a different one. This is an intriguing challenge for future experimentation.

One salient difference between these channels is their voltage dependence. Martinac *et al.* and Cui *et al.* have shown that the open probability of MscS is increased on depolarization (*5, 6*). The voltage dependence is small, but this is the first structure of an ion channel modulated by voltage and thus can give us interesting pointers on how a structure may achieve voltage dependence. TM1 and TM2 have arginines near the intracellular side of the membrane region. These arginines could act as voltage sensors. A hyperpolarization would tend to pivot TM1 and TM2 to make them more perpendicular to the membrane (the closed state, see the figure), whereas a depolarization would pivot the intracellular sides of those helices away from the center of symmetry, increasing their tilt (the open state, see the figure). Indeed, a change of only 18° in the tilt would produce a small charge translocation that, multiplied by seven, would account for the measured voltage dependence in spheroplasts.

In the MscS structure, arginines 46 and 74 in TM1 and TM2 point to the bilayer, and it is difficult to reconcile this orientation with a net charge that can be oriented by the electric field, unless the charge is highly solvated. However, the resolution of the structure does not specify the exact position of these side chains, so they may be located between the helices and exposed to water crevices, presumably connected to the intracellular solution. This is reminiscent of the gating charges in sodium and potassium channels, where the arginines and lysines of the S4 segment reside in an internally connected water crevice in the closed state and an externally connected crevice in the open state, so that a depolarization effectively moves the charge from the inside to the outside (*15*). The conformational change induced by voltage in the sodium and potassium channels is not known, but involves rotation, tilting, and perhaps a small amount of translation; it is not even known whether the S4 segment is indeed α-helical. In MscS we have a crystal structure that includes what is likely a bona fide voltage sensor. This structure is the first step on the path toward a molecular description of the structural changes induced by voltage in membrane proteins.



Sensing tension and voltage. (**A**) Comparison of MscL (left) and MscS (right) stretch-activated channels. The channels have been sliced along their centerline, and colors code for the different structural elements in each molecule. (**B**) A possible mechanism of transduction and gating in MscS. The channel is represented in its open conformation (with basal tension) as determined by Bass *et al.* (*4*). The transmembrane segments 1 to 3 of subunits 1 and 4 as well as arginines 46 and 74 have been enhanced. Pore closing (blue arrows) might be induced by releasing the tension (white arrow) or by hyperpolarization (black arrow) that drives arginines 46 and 74 toward the inside surface.

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