# Molecular Identification of a Mechanosensitive Channel in Archaea

Anna Kloda and Boris Martinac
Department of Pharmacology, QEII Medical Center, The University of Western Australia, Nedlands WA 6907, Australia

ABSTRACT The TM1 domain of the large conductance mechanosensitive (MS) channel of *Escherichia coli* was used as a genetic probe to search the genomic database of the archaeon *Methanoccoccus jannashii* for MscL homologs. We report that the hypothetical protein MJ0170 of *M. jannashii* exhibited 38.5% sequence identity with the TM1 domain of Eco-MscL. Moreover, MJ0170 was found to be a conserved homolog of MscS, the second type of *E. coli* MS channel encoded by the *yggB* gene. Furthermore, we identified a cluster of charged residues KIKEE in the C-terminus of MJ0170 that strikingly resembled the charged C-terminal amino acid cluster present in Eco-MscL (RKKEE). We cloned and expressed MJ0170 in *E. coli,* which when reconstituted into liposomes or expressed in the cell membrane of giant *E. coli* spheroplasts, exhibited similar activity to the bacterial MS channels. Our study suggests that the *M. jannashii* MS channel and its homologs evolved as a result of gene duplication of the ancestral MscL-like molecule with the TM1 domain remaining the most conserved structural motif among prokaryotic MS channels.

## INTRODUCTION

Traditionally ion channels have been studied in the context of neurophysiology and within eukaryotic metazoan preparations (Hille, 1992). With the advent of the patch-clamp recording technique (Hamill et al., 1981), studies of ion channels in various microbes including prokaryotes gained momentum. Mechanosensitive (MS) ion channels have been extensively studied in both Gram-negative and Gram-positive bacteria (Zoratti and Ghazi, 1993; Martinac et al., 1992; Martinac, 1993; Blount et al., 1999). The existence of MS ion channels in cell membranes of Archaea, the third domain of the universal phylogenetic tree (Woese, 1994; Stein and Simon, 1996; Pace, 1997) was first documented in the archaebacterium *Haloferax volcanii* (formerly *Halobacterium volcanii*) (Le Dain et al., 1998). As a distinct group of prokaryotic microorganisms archaebacteria comprise several different families of cells adapted to extreme environments such as super-hot ocean hydrothermal vents or the high salt concentrations found in the Dead Sea (Barinaga, 1994). The existence of MS channels in archaeal and bacterial cell membranes suggests that this class of ion channels might have appeared very early during the evolution of life on Earth (Garcia-Añovernos and Corey, 1997; Martinac, 1999).

The TM1 domain of the bacterial MS ion channel of large conductance (MscL) is highly conserved among Gram-negative and Gram-positive bacteria (Sukharev et al., 1997; Moe et al., 1998; Batiza et al., 1999; Spencer et al., 1999; Oakley et al., 1999). In this study we used the TM1 domain of MscL of *Escherichia coli* (Eco-MscL) as a genetic probe to screen the genomic database of *Methanococcus jannashii* (Bult et al., 1996) for MscL homologs in Archaea. We identified the hypothetical protein (MJ0170) as a putative MS channel with a high degree of homology to Eco-MscL. On alignment of the sequences of MJ0170 and the recently cloned *E. coli* MS channel of the small conductance (Eco-MscS) (Levina et al., 1999), we found a high degree of homology between these two proteins. This approach has allowed recognizing and establishing the mutual relationship and common evolutionary origin of the new family of prokaryotic (bacterial and archaeal) MS channels.

*Received for publication 21 July 2000 and in final form 11 October 2000.*

Address reprint requests to Dr. Boris Martinac, Department of Pharmacology, QEII Medical Center, The University of Western Australia, Nedlands, WA 6907, Australia. Tel.: 61-8-9346-2986; Fax: 61-8-9346-3469; E-mail: bmartinac@receptor.pharm.uwa.edu.au.



0006-3495/01/01/229/12 $2.00

## MATERIALS AND METHODS

### Bacterial strains and culture conditions

*E. coli* cells harboring the AMJ BZ56 clone encoding the MJ0170 protein were obtained from American Type Culture Collection (Rockville, MD). *E. coli* strain M15 (pREP4::kan) (Qiagen, Chatsworth, CA) was used as a host for a recombinant plasmid harboring the MJ0170 gene. *E. coli* strain MJF465 of the following genotype Frag 1, Δ*mscL*::Cm, Δ*yggB*, Δ*kefA*::kan (Levina et al., 1999) was used for protein expression and electrophysiology. Strains were grown at 37°C in Luria-Bertani broth (LB) containing 10g/L Bacto-tryptone, 5g/L yeast extract, and 5g/L NaCl supplemented with ampicillin (100 μg/ml), chloramphenicol (20 μg/ml), and kanamycin (25 μg/ml), according to the selection requirements. The gene expression was induced with isopropyl-1-thio-β-D-galactopyranoside (IPTG) once the cell culture reached mid-log phase ($OD_{600}$ of ~0.6).

### Cloning and protein purification

The TM1 domain of Eco-MscL was used as a genetic probe to search the *M. jannaschii* genomic database for a putative MscL homolog. The sequence alignment was generated using SIM Alignment Tool for Protein Sequences available at the ExPasy Molecular Biology server. The alignment that produced the highest score was used to generate either the Kyte-Doolittle hydropathy plot or the dense alignment surface (DAS) plot for transmembrane segments prediction (ExPasy) to ensure the newly identified protein had the properties of a membrane protein. The entire open reading frame of *MJ0170* was amplified by polymerase chain reaction (PCR) using the AMJBZ56 clone as a template and cloned into pQE-32 expression vector (Qiagen) as a *BamHI-SalI* fragment using standard

cloning procedures (Sambrook et al., 1989). The 6xHis MJ0170 recombinant protein was purified as previously described (Sukharev et al., 1999).

## Liposome preparation, protein reconstitution, and spheroplast preparation

The MJ0170 protein was reconstituted into liposomes according to the methods described previously for MS ion channels in *E. coli* and *H. volcanii* (Delcour et al., 1989; Sukharev et al., 1993; Häse et al., 1995; Le Dain et al., 1998). Bilayer blisters of 50–100 $\mu$m in diameter, which appeared after incubation of the proteoliposomes in the recording solution (Delcour et al., 1989), were examined using the patch-clamp technique.

For spheroplast preparation, bacterial cells AMJ465 harboring the plasmid pQE-32MJ0170 were cultured to mid-log phase as described for protein purification experiments and induced with IPTG for 30 min. Spheroplasts were then prepared according to the previously described method (Martinac et al., 1987).

## Cell-free expression

The TNT Quick Coupled Transcription/Translation system was used for transcription and translation of *MJ0170* gene cloned downstream from SP6 RNA polymerase promoter of the pSP64 poly-A vector. For protein labeling, 25-$\mu$l reactions containing 0.5 $\mu$g of either pSP64-polyA MJ0170 construct or empty vector were set up with 40 $\mu$l of TNT mix of reticulocyte lysate, SP6 polymerase, a complement of amino acids without methionine, and Rnasin (RNAse inhibitor). The reactions were supplemented with 2 $\mu$l of [$^{35}$S]methionine (10 mCi/ml) and 2.5 $\mu$l of canine pancreatic microsomes (Sukharev et al., 1994). For details see also Promega Technical Bulletin 126 (Promega, Madison, WI). After 2 h of incubation at 30°C the reactions were heated at 100°C for 5 min in the presence of sample buffer (6% 32 $\beta$-mercaptoethanol, 3% SDS, 0.3% bromophenol blue, and 1% glycerol) and separated on 12% SDS-PAGE gel. The gel was fixed in a solution containing 10% acetic acid plus 30% methanol, dried under vacuum, and exposed to x-ray film. For functional assay of channel activity, 50-$\mu$l reactions containing either pSP64-polyA MJ0170 construct or empty pSP64-poly A vector were set up with a full complement of unlabeled amino acids under conditions described above. Microsomal membranes were washed in KCl buffer (200 mM KCl, 10 mM HEPES-KOH, pH 7.0), and the membrane pellet was resuspended in 50 $\mu$l of liposome suspension containing 2 mg of phosphatidylcholine supplemented with 10% cholesterol in 10 mM MOPS buffer, pH 7.2. Droplets of mixed suspension were subjected to a dehydration-rehydration cycle described previously (Delcour et al., 1989; Häse et al., 1995), and proteoliposomes were examined for the channel activity using the patch-clamp technique.

## Electrophysiological recording

Single-channel currents were filtered at 2 kHz, digitized at 5 kHz, and analyzed using pCLAMP6 data acquisition and analysis software (Axon Instruments, Foster City, CA). Current recordings were viewed by the Axoscope for Windows program (Axon Instruments). Current amplitudes were determined by measuring the difference between the cursor aligned at peak and baseline currents. Channel conductance was estimated from current voltage plots. Suction applied to the patch-clamp pipette was measured by the piezoelectric pressure transducer (Omega Engineering, Stamford, CT).

## Estimate of the MS channel free energy of activation from Boltzmann distribution

Open probability of the MscMJ channels plotted against the negative pressure (suction) applied to the patch pipette was fitted to a Boltzmann distribution function of the form $NP_o = NP_{o_{max}}[1 + \exp\alpha(p_{1/2} - p)]^{-1}$, where $N$ is the unknown number of channels in the patch, $P_o$ is the open probability, $P_{o_{max}}$ is the maximum open probability, $p$ is the negative pressure applied to the patch pipette, $p_{1/2}$ is the pressure at which the open probability is 0.5, and $\alpha$ is the channel sensitivity to pressure. The values for $p_{1/2}$ and $1/\alpha$ of MscS estimated in giant spheroplasts of *E. coli* were 36 $\pm$ 23 mm Hg and 5 $\pm$ 1 mm Hg ($n$ = 9), respectively (B. Martinac, unpublished results). The single-channel open probability was estimated from the total current divided by the single-channel current giving $NP_o$ and divided by the maximum number of channels observed in the patch. By using a two-state Boltzmann model with the change of area $t\Delta A$ being the dominant energy term (Sukharev et al., 1999), it follows according to the model of Howard et al. (1988) that the free energy $\Delta G$ is a linear function of membrane tension $t$; i.e., $\Delta G = t\Delta A - \Delta G_o$, 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 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 a MS channel open by external mechanical force at the open probability of $0 < P_o < 1$. The Boltzmann function for the open probability of a single MS channel can be written as $P_o/(1 - P_o) = \exp[\alpha(p - p_{1/2})] = \exp[(t\Delta A - \Delta G_o)/kT]$. Because membrane tension $t$ is nearly proportional to the pressure within the range applied to the patch pipette in this study, it is well approximated by a modified form of the Laplace's law, such that $t - t_{1/2} = (p - p_{1/2})(r/2)$, where $r$ is the radius of curvature of the liposome membrane patch under external negative pressure $p$ applied to the patch pipette. Thus, it follows that when the open probability $P_o = 0.5$ (i.e., $p = p_{1/2}$ and $t = t_{1/2}$) the free energy difference $\Delta G = 0$. Consequently, $t_{1/2} = \Delta G_0/\Delta A$ and $p_{1/2} = 2\Delta G_0/r\Delta A$, whereas $\alpha = r\Delta A/2kT$.

# RESULTS

## Multiple sequence alignment and phylogeny of prokaryotic MS channels

We considered two types of alignments of MJ0170 (referred to as MscMJ), the local alignment against *E. coli* MscL (Fig. 1 *A*) and the global alignment against identified MscL and MJ0170 homologs (Fig. 2 *A*). The local alignment identified MscL-like motifs TM1, TM2, and TM1-loop, which were preserved within the first, the second, and the third helical domains of MJ0170, respectively, with the following scores: 1) 38.5% identity in the stretch of 26 residues corresponding to the TM1 transmembrane helix of Eco-MscL and the first putative membrane-spanning domain of MJ0170, 2) 31.8% identity in the 22-residue overlap corresponding to most of the TM2 transmembrane helix of Eco-MscL and the second putative membrane-spanning domain of MJ0170, and 3) 40% identity in 20 amino acid residues encompassing a section of the TM1 helix plus the periplasmic loop of Eco-MscL and a section of the third putative transmembrane domain of MJ0170. Furthermore, the MJ0170 amino acid sequence could be aligned against the sequence of the YggB protein underlying the activity of Eco-MscS, the bacterial MS channel of small conductance (Levina et al., 1999). This alignment exhibited 28% identity in the overlapping 226 residues of the YggB sequence encompassing the TM1-periplasmic loop region of MscL and the third putative transmembrane helix and C-terminal portion of MJ0170. The alignment demonstrated that the

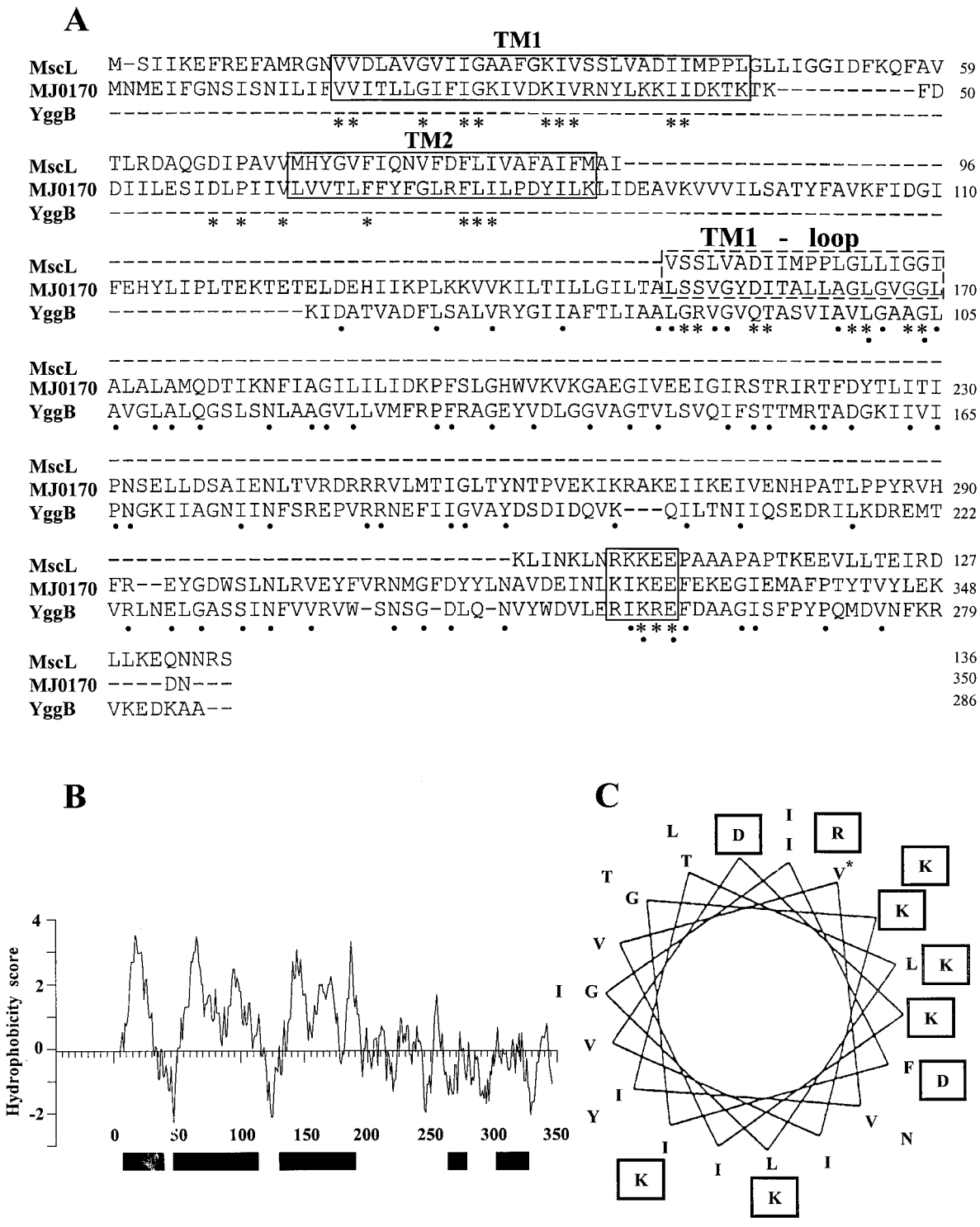
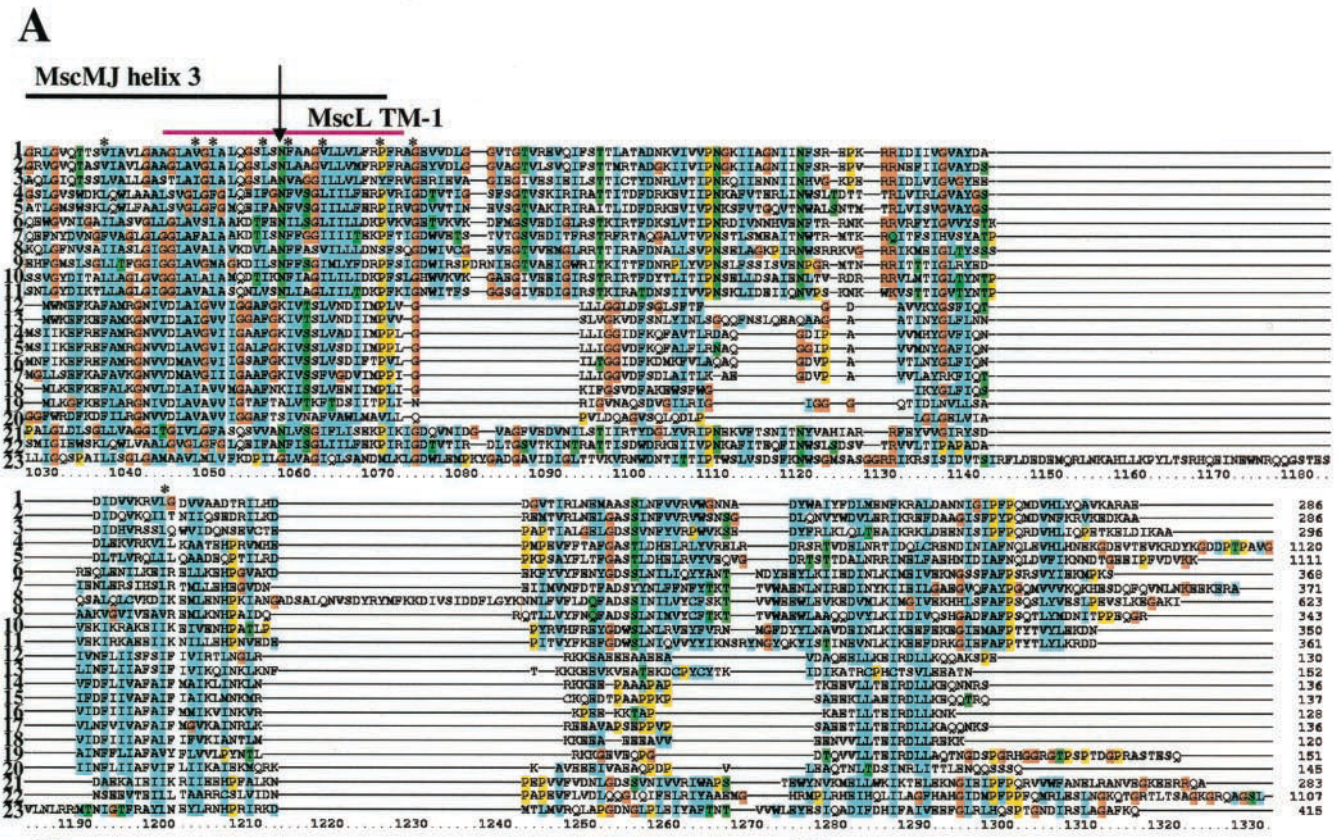
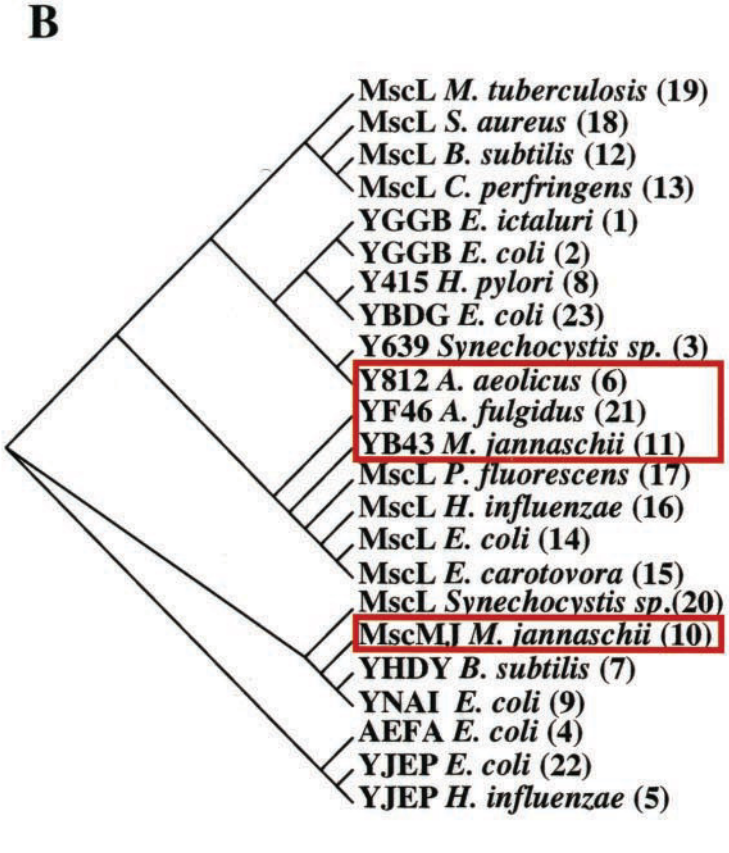
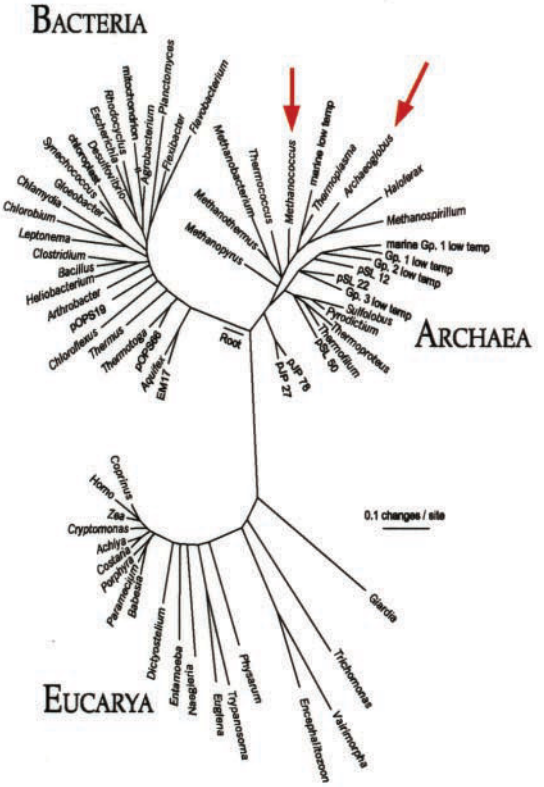



FIGURE 1 Structural properties of the MJ0170 (MscMJ) mechanosensitive channel protein. (*A*) Regions of homology between MJ0170, MscL, and YggB protein sequences. The identical residues found in both the MscL and MJ0170 amino acid sequences are marked with an asterisk, whereas those common to the YggB and MJ0170 sequences are marked with filled circles. TM1 and TM2 MscL transmembrane domains that match MJ0170 homolog sequences are boxed. (*B*) Hydropathy profile and predicted $\alpha$-helical secondary structure (black bars) of MJ0170. Positive numbers indicate relative hydrophobicity in the plot. (*C*) Helical wheel representation of the putative first transmembrane domain TM1 of MJ0170 reveals its amphipathic character. Charged residues are boxed. The start of the helix is marked with an asterisk.


A
MscMJ helix 3
MscL TM-1


B
MscL M. tuberculosis (19)
MscL S. aureus (18)
MscL B. subtilis (12)
MscL C. perfringens (13)
YGGB E. ictaluri (1)
YGGB E. coli (2)
Y415 H. pylori (8)
YBDG E. coli (23)
Y639 Synechocystis sp. (3)
Y812 A. aeolicus (6)
YF46 A. fulgidus (21)
YB43 M. jannaschii (11)
MscL P. fluorescens (17)
MscL H. influenzae (16)
MscL E. coli (14)
MscL E. carotovora (15)
MscL Synechocystis sp.(20)
MscMJ M. jannaschii (10)
YHDY B. subtilis (7)
YNAI E. coli (9)
AEFA E. coli (4)
YJEP E. coli (22)
YJEP H. influenzae (5)


C
BACTERIA
ARCHAEA
EUCARYA
Root
0.1 changes / site

first, part of the second, and the third membrane-spanning domain of MJ0170 shared high homology with MscL, whereas the large portion of the MJ0170 sequence starting at the third transmembrane helix and including most of the C-terminus shared high homology with the YggB protein. In addition, a cluster of charged residues is conserved in the C-terminal domains of all three proteins, MscL (RKKEE), YggB (RIKRE), and MJ0170 (KIKEE) (Fig. 1 *A*). This finding is of potential significance, as MscL activity was abolished in a mutant having 33 C-terminal residues that included the charged cluster RKKEE deleted (Blount et al., 1996; Häse et al., 1997). Interestingly, a charged cluster with a very similar sequence RVISKKTKEE is also present in the C-terminal domain of the mammalian mechanogated potassium channel TREK-1 (Patel et al., 1998).

Multiple sequence alignment revealed 1) a high degree of preservation of all three helical domains of MJ0170 among its homologs (data not shown) and 2) a recognizable consensus within the alignment of MscMJ and MscL homologs (Fig. 2 *A*). MscL homologs align to the C-terminal portion of MJ0170 homologs with prominent conservation of the MscL TM1 motif within the distal portion of the third transmembrane helix of MJ0170. Glycine residues separated by three to four hydrophobic residues form the signature sequence of this region among all aligned proteins. Furthermore, proline and glycine residues at the MscL TM1-periplasmic loop interface are highly conserved among nearly all aligned proteins. Interestingly, it was demonstrated that proteolytic cleavage of the MscL periplasmic loop led to a dramatic increase in channel pressure sensitivity (Ajouz et al., 2000), possibly indicating the importance of the conserved residues in controlling the channel mechanosensitivity.

The phylogenetic tree of aligned sequences revealed a common origin of MS channels (Fig. 2 *B*) in cells belonging to both prokaryotic (archaeal and bacterial) domains of the universal tree of life (Fig. 2 *C*). Sequences are clustered into three main branches on the basis of their similarity, which reflects their common ancestry and evolutionary divergence over time. Two of the main branches consist of a mixture of MscL and MJ0170 homologs whereas MscL is absent from the third branch. The proteins on the branch with no MscL are approximately four times the size of MJ0170 homologs and have preserved MJ0170-like rather than the MscL-like structural motif indicating their more recent origin.

## Secondary structure analysis

Hydropathy plot analysis combined with the secondary structure prediction (Fig. 1 *B*) indicated that MJ0170 had three membrane-spanning domains followed by a very large hydrophilic C-terminal tail. Moreover, the helical wheel analysis of the putative TM1 helix of MJ0170 indicated that the helix is amphipathic in character (Fig. 1 *C*) and may therefore constitute a structural part of the channel pore. Because it has been suggested that glycosylation in thermophilic Archaea serves to stabilize proteins at high temperatures (Voorhorst et al., 1997), we searched for putative glycosylation sites in MJ0170 using the NetOGlyc 2.0 program. We identified threonine 283 as a high-probability O-glycosylation site, which together with the hydropathy plot indicated that MJ0170 belonged to the type VI of membrane proteins having an odd number of membrane-spanning helices with the N-terminus located in the cytoplasm and the C-terminus in the extracellular space (Reithmeier and Deber, 1992).

## Mechanosensitivity of MscMJ

To determine whether MJ0170 is a mechanosensitive protein we amplified the entire open reading frame of the MJ0170 gene by PCR, cloned it into an expression vector, and expressed it in *E. coli* as a 6xHis-tagged recombinant protein (see Materials and Methods). The SDS-PAGE analysis showed that MJ0170 protein ran as a 37-kDa band (Fig. 3 *A*, *left*). The purified protein was reconstituted into liposomes and found to exhibit MS-channel-type activity characterized by long openings when examined by the patch-clamp technique (Fig. 3 *A*, *right*). MJ0170 exhibited similar MS channel activity when expressed and examined by patch

FIGURE 2 Phylogeny of prokaryotic MS channels. (*A*) Multiple sequence alignment of MscMJ and MscL homologs (Clustal X 1.8). The alignment includes the C-terminal portion of MscMJ and its homologs starting from helix 3 (marked with a horizontal black line) and the complete sequences of MscL homologs. The colored residues share similarity with the consensus, which is defined by the maximized alignment of all sequences. Yellow represents proline residues, brown represents glycine residues or its divergent substitution with basic residues, green is assigned to polar residues, whereas blue represents hydrophobic residues or their divergent substitutions with mostly acidic residues (see Clustal X 4 color index for more information on the color scheme chosen for this figure). Highly conserved residues are marked with an asterisk. The most extensive consensus sequence between all aligned proteins is marked by the start of the helix 3 of MscMJ (black horizontal line) and TM1 domain of MscL (purple horizontal line). The arrow points to a highly conserved asparigine residue (N182) within the helix 3 of MscMJ and its homologs, which is replaced with lysine in *E. coli* MscL (K31) and most of its homologs. The sequences are numbered and their names given in *B*. (*B*) Phylogenetic tree of complete aligned sequences of MscMJ homologs and MscL homologs showing the common ancestry of prokaryotic MS channels. The archaeal homologs of MscMJ are boxed (red). Numbers in parentheses correspond to the sequence number in *A*. (*C*) Universal phylogenetic tree derived from comparative sequencing of 16 S ribosomal RNA (modified from Pace, 1997, with permission). MscMJ homologs are found in two major domains of life: bacteria and archaea. The red arrows point to archaeal phyla of *Archaeoglobus* and *Methanococcus* with known genomic DNA sequences, which were found to harbor MscMJ homologs.

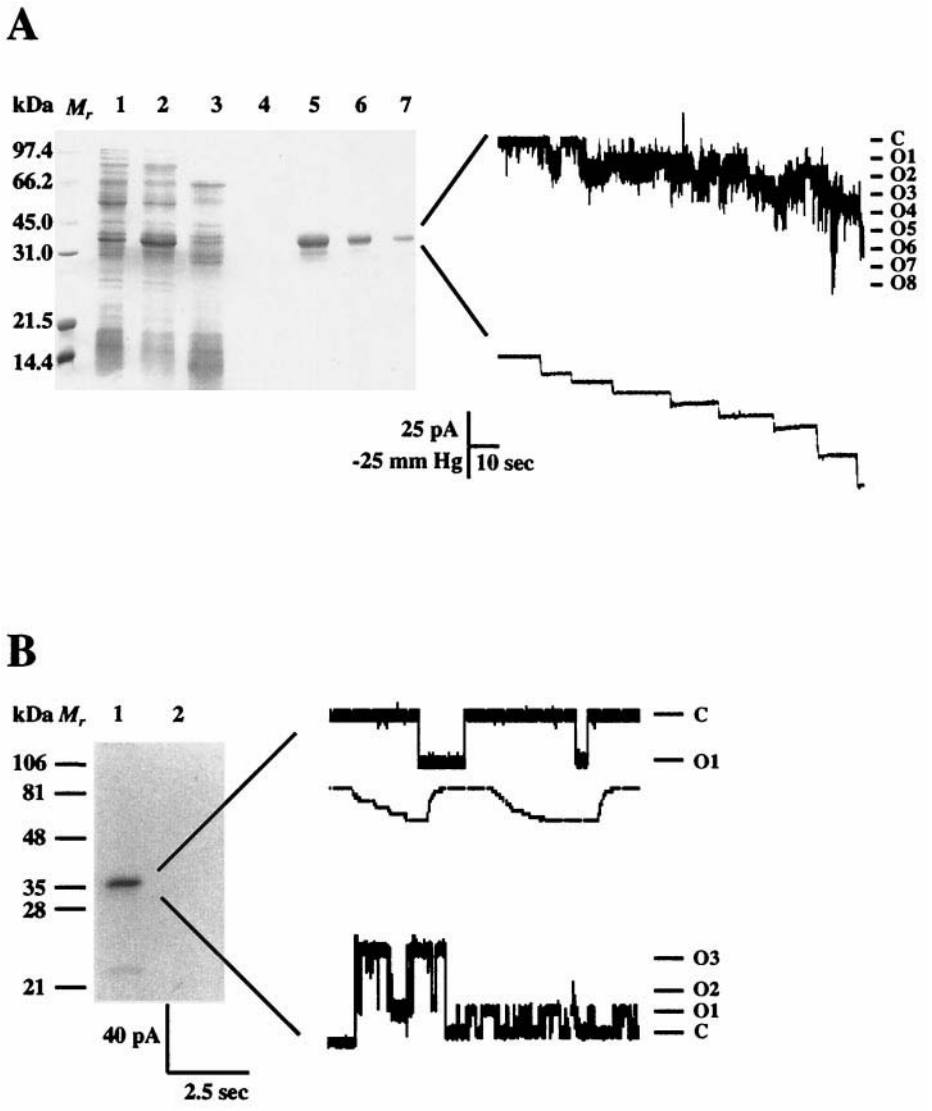



FIGURE 3 Molecular identification of MscMJ. (*A*, *left*) SDS-PAGE of the 6xHis MJ0170 protein expressed in *E. coli.* Lanes 1 to 7 indicate protein pattern before (*lane 1*) and after (*lane 2*) induction with IPTG, flow through a Ni-NTA column (*lane 3*), 20 mM imidazole wash (*lane 4*), and the first, second, and third 250-mM elution fraction (*lanes 5*, *6*, and *7*, respectively). (*A, right*) Activation of the purified recombinant MJ0170 protein reconstituted into azolectin liposomes by negative pressure applied to the patch pipette. Upper trace shows single-channel current fluctuations recorded at −50-mV pipette voltage. C denotes the closed state and $O_n$ denotes the open state of *n* number of channels in the particular liposome patch. Lower trace shows the negative pressure applied to the patch pipette. (*B*) Cell-free expression of the MJ0170 protein. The autoradiogram of the SDS-PAGE on the left shows the expression of the MJ0170 protein band in the lysate driven by a *mj0170*-containing vector (*lane 1*) or no protein expression in the control lysate containing the empty vector (*lane 2*). Activities of the MS channels were recorded in a liposome patch containing a membrane fraction of the expression lysate at +40-mV pipette voltage without applied negative pressure (*lower trace*) and at −30 mV with applied pressure (*upper trace*). Closed and open states of the channels are marked C and O, respectively.

clamp in giant spheroplasts of *E. coli* (data not shown) (Martinac et al., 1987).

In addition, we expressed the MJ0170 protein in vitro in a cell-free (rabbit reticulocyte lysate) transcription/translation system. This system proved to be successful with heterologous expression of MscL gene and was reported to be devoid of ion channel activities (Sukharev et al., 1994) in accordance with our control experiments that contained empty vector. Autoradiographic analysis showed that the protein runs as a band of similar size (Fig. 3 *B*, *left*) to that of the protein expressed in the *E. coli* membrane (Fig. 3 *A*, *left*). The MJ0170 protein expressed in the cell-free system exhibited MS channel activity when reconstituted into liposomes. Two types of conductances were observed: the smaller conductance corresponded to that of the protein expressed in *E. coli* and the larger approximately three times its size (Fig. 3 *B*, *right*). At present, we do not know the cause for the appearance of the MS channel with larger conductance, but it could be that in the cell-free system the channel might have formed an oligomer of a larger number of subunits than in the *E. coli* membrane. Furthermore, some difference in the channel conductance might have originated from the post-translational modifications of the protein, such as glycosylation in the cell-free expression system (Technical Manual TM231 and Technical Bulletin 126, Promega). Because the large conductance events were observed exclusively in the cell-free expression system, the remaining analysis in this study was confined to the small conductance events.

When plotted against the negative pressure the open probability of the MJ0170 MS channel could be fitted to a Boltzmann distribution function (Fig. 4 *A*), which demonstrated that the MJ0170 channel protein is gated by membrane tension. Consequently, we propose to rename the hypothetical protein MJ0170 as MscMJ, for mechanosensitive channel of *M. jannashii.* The results from four different

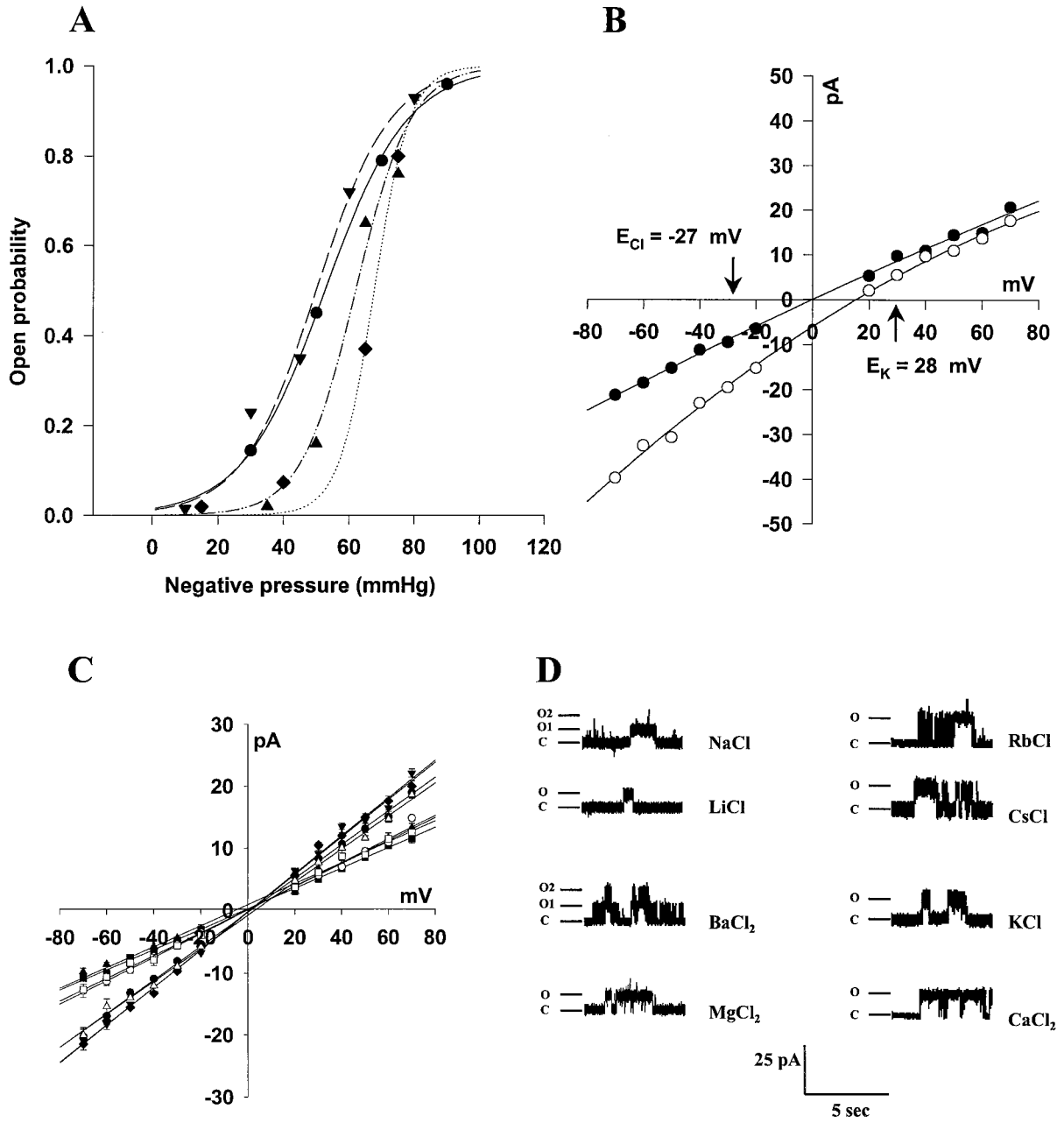



FIGURE 4 Mechanosensitive and conductive properties of the MJ0170 MS channel protein. (*A*) The open probability of the MscMJ channel from four different patches was plotted against the negative pressure (suction) applied to the patch pipette and fitted to a Boltzmann distribution function. The channel sensitivity to pressure $1/\alpha$ varied between 9 and 13 mm Hg per e-fold change in open probability, and the pressure required for half-activation $p_{1/2}$ (where $P_o = 0.5$) varied between 50 and 64 mm Hg. Single-channel open probability versus negative pressure was obtained by dividing open probability of multiple channels ($NP_o$) by the number of active channels in the particular patch ($N$) obtained from the best fit of the plot $\ln[NP_o/(NP_{o_{max}} - NP_o)]$. Saturating dose responses were obtained in two patches. In the remaining two patches, 80% of the single-channel open probability was achieved. The pressures at which patch lysis occurred were not used for analysis. (*B*) Current-voltage relationship for MscMJ. Single-channel conductance calculated from the current-voltage plot obtained in 200 mM KCl, 5 mM $MgCl_2$, and 5 mM HEPES (pH 7.2) buffer was 270 pS. The reversal potential was obtained for the same patch by fitting a second-order polynomial curve to the current-voltage relation acquired after replacing the bath solution with the buffer containing 600 mM KCl, 5 mM $MgCl_2$, and 5 mM HEPES (pH 7.2) was 18 mV, which indicated the channel preference for cations over anions. The ionic strength and osmolarity were both changed threefold. The reversal potentials for chloride ($E_{Cl}$) and potassium ($E_K$) are indicated by arrows. (*C*) Ionic selectivity of MscMJ. Closed symbols represent monovalent cations: $K^+$ (●), $Na^+$ (■), $Li^+$ (▲), $Rb^+$ (♦), and $Cs^+$ (▼). Open symbols represent divalent cations: $Ba^{2+}$ (○), $Mg^{2+}$ (□), and $Ca^{2+}$ (△). Each data point is mean ± SE of at least three experiments ($n = 3$). (*D*) Current traces of MscMJ recorded at +40-mV pipette voltage in different buffers containing either 200 mM XCl or 200 mM $YCl_2$ plus 40 mM $MgCl_2$, 5 mM HEPES (pH 7.2), where X and Y symbolize monovalent and divalent cations, respectively. Negative pressures applied to activate the channels were in the range between 50 and 100 mm Hg.

patches showed that the sensitivity of MscMJ to pressure $1/\alpha = 11 \pm 2$ mm Hg per e-fold change in the channel open probability, whereas the pressure required for 50% open probability $p_{1/2} = 57 \pm 6$ mm Hg.

Voltage of either sign not only caused an increase in the channel opening probability but also affected the channel kinetics characterized by long openings at voltages ≤90 mV and very brief openings at voltages ≥90 mV (data not shown). It is likely that closely clustered positive charges of four lysines and one arginine on one side of the amphipathic TM1 helix (Fig. 1 *C*) may confer the voltage sensitivity to the channel.

**TABLE 1 Ion selectivity of MscMJ**

| Ion | Conductance (pS) | *N* | $P_X/P_K$ |
|---|---|---|---|
| $Li^+$ | 168 ± 7 | 3 | 0.6 |
| $Na^+$ | 169 ± 6 | 3 | 0.6 |
| $K^+$ | 271 ± 4 | 5 | 1.0 |
| $Rb^+$ | 306 ± 4 | 7 | 1.1 |
| $Cs^+$ | 303 ± 7 | 7 | 1.1 |
| $Mg^{2+}$ | 184 ± 2 | 4 | 0.7 |
| $Ba^{2+}$ | 180 ± 4 | 6 | 0.7 |
| $Ca^{2+}$ | 259 ± 8 | 5 | 1.0 |

$P_X/P_K$ (where X = other ions) is the calculated ratio of the conductance of the channel (i.e., $P_X/P_K = g_X/g_K$) in solutions containing 200 mM of the particular ion X to the conductance calculated for 200 mM potassium.

## Ionic selectivity

Next, we also estimated the conductance and selectivity of MscMJ (Fig. 4, *B–D*). In a symmetric buffer containing 200 mM KCl plus 5 mM $MgCl_2$ the channel had a conductance of $g = 270 \pm 19$ pS ($n = 5$). When the bath solution was exchanged to 600 mM KCl plus 5 mM $MgCl_2$ the reversal potential $E_{rev}$ shifted ~18 mV toward the reversal potential for potassium. From six different experiments we obtained $E_{rev} = 18 \pm 2$ mV. Using this value we calculated the permeability ratio of MscMJ for potassium versus chloride of $P_K/P_{Cl} \approx 6$, which indicated a preference of the channel for cations over anions (Fig. 4 *B*). Thus, MscMJ selectivity differs from the one of MscS, which was shown to have a slight preference for anions over cations with $P_{Cl}/P_K \approx 3$ (Martinac et al., 1987). When the channel selectivity among mono- and divalent cations was examined the selectivity sequence of MscMJ for monovalents corresponded to the Eisenman sequence I (Hille, 1992) suggesting that a weak anionic site formed a selectivity filter in the channel pore. The following preference for monovalent cations was obtained from the current-voltage plots: $Cs^+ = Rb^+ > K^+ > Na^+ = Li^+$. For divalent cations the sequence was $Ca^{2+} > Ba^{2+} = Mg^2$. Selectivity for $Cs^+$ and $Rb^+$ was significantly different from the channel selectivity for $K^+$ and $Ca^{2+}$ ($p < 0.05$, ANOVA). Similarly, selectivity for $Ba^{2+}$ and $Mg^{2+}$ differed significantly from the selectivity for $Na^+$ and $Li^+$ ($p < 0.05$). Overall, $Cs^+ = Rb^+ > Ca^{2+} = K^+ > Ba^{2+} = Mg^{2+} > Na^+ = Li^+$. Interestingly, among divalent cations calcium was the most permeant ion (Fig. 4, *C* and *D*, and Table 1).

## Effect of amphipaths on MscMJ

Cationic chlorpromazine (CPZ) and anionic trinitrophenol (TNP) were shown to activate reversibly MscS in giant spheroplasts of *E. coli* (Martinac et al., 1990). Therefore, we examined the effect of the two amphipaths on MscMJ (Fig. 5, *A* and *B*) (note that MscMJ refers only to small conductance events; activation of larger conductance events by the amphipaths was never observed in this study). The activation of the MscMJ channels both by CPZ as well as by TNP occurred within tens of minutes, comparable to the activation of MscS by the two amphipaths. This result suggested a similar mechanism of activation for both the bacterial and archaeal MS channel via preferential insertion of CPZ and TNP into one of the two monolayers of the lipid bilayer of the spheroplast membrane.

## Effect of expression of MscMJ on *E. coli* growth

Because it has been shown that MscL and MscS function as safety valves in osmotically challenged bacteria (Levina et al., 1999) we examined the effect of expression of MscMJ on the growth of *E. coli* cells (Fig. 6). *E. coli* growth was significantly reduced when expression of *mscMJ* was induced with IPTG compared with noninduced cell culture. The effect of the *mscMJ* expression was partially reversed when the cells were grown in media of higher osmolarity containing either 300 mM NaCl, 300 mM KCl, or 600 mM sorbitol compared with standard LB media ($p < 0.05$, ANOVA).

## DISCUSSION

In search of an archaeal homolog of *E. coli* MscL we identified and cloned a gene from the archaeon *M. jannaschii* using the TM1 domain of *E. coli* MscL as a genetic probe against the *M. jannaschii* genome. Furthermore, we demonstrated that the newly identified archaeal MS channel that we named MscMJ shares a common ancestral origin with two types of prokaryotic MS ion channels, MscL and MscS. Phylogenetic evidence presented in this study suggests that prokaryotic MS channels may have originated from an *mscL*-like molecule via gene duplication of the ancestral progenitor gene followed by divergence. This implies that bacterial MS channels are most likely the evolutionary predecessors of the archaeal MS channels in accordance with the present organization of the phylogenetic tree (Pace, 1997).

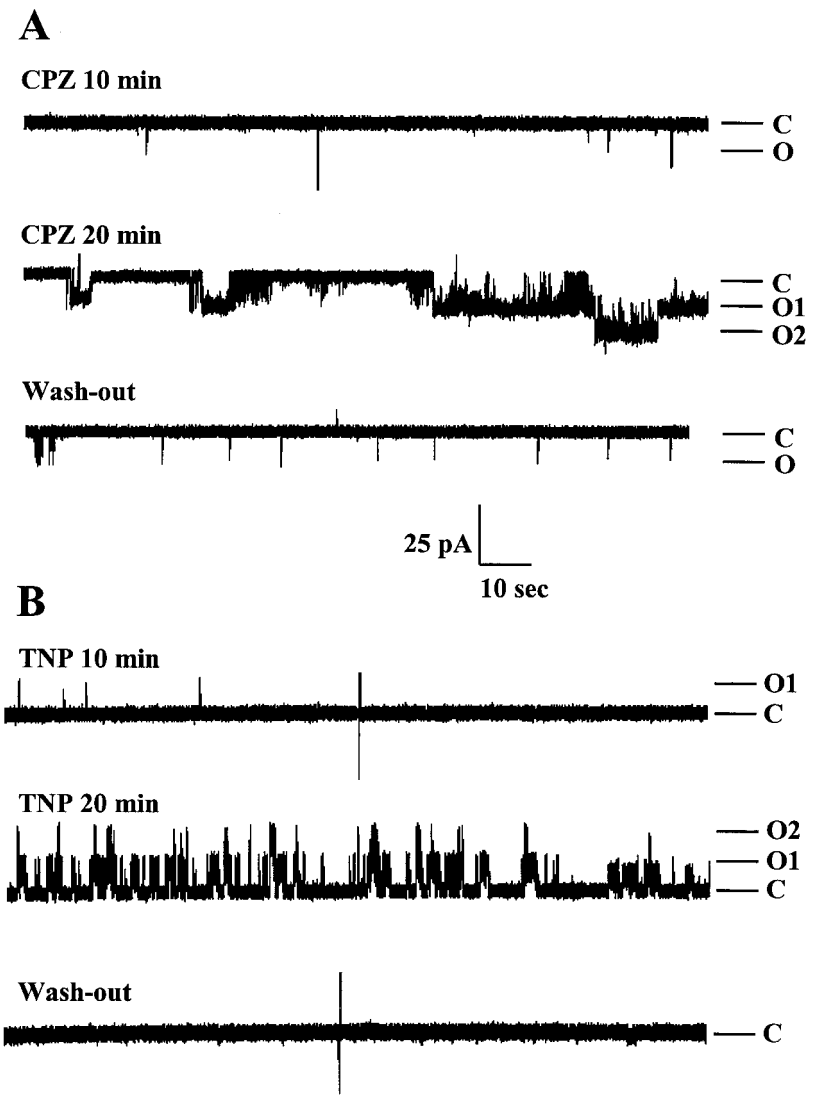



FIGURE 5 Activation of MscMJ by amphipaths. MscMJ channels were activated by amphipaths in inside-out excised patches of *E. coli* giant spheroplasts. The 20 $\mu$M chlorpromazine (CPZ; *A*) and 500 $\mu$M trinitrophenol (TNP; *B*) reversibly activated the channels. Recordings were obtained at 10 and 20 min after the amphipaths were added to the bath solution. Pipette voltage was held at −50 mV and +50 mV and pressure was −50 mm Hg and −80 mm Hg during the CPZ and TNP experiments, respectively.

## Evolution of prokaryotic MS channels

Comparative sequence analysis of known archaeal genes with the genetic makeup of bacterial and eukaryotic lines of descent revealed that some genes, especially those involved in replication, transcription, and translation, resemble eukaryotic genes. However, genes whose function is related to energy production, cell division, and metabolism are similar to those found in bacteria. Furthermore, *M. jannaschii* genes that control the transport of inorganic ions such as potassium and sodium across the cell membrane are very bacteria-like, indicating that the ion transport pathway was derived from a common ancestor (Morell, 1996). In addition, MS channels have been implicated to play a role in the regulation of turgor pressure, which is essential for division and growth of bacterial cells (Csonka and Epstein, 1996). Consequently, it would be expected that archaeal MS channels are more closely related to bacterial rather than eukaryotic MS channels, which again indicates their common heritage.

Several findings in our study support the idea of the evolution of prokaryotic MS channels via duplication of an MscL-like molecule. Sequence alignments used in this study indicated that two MscL-like TM1 structural motifs have been preserved during evolution within the sequence of MscMJ, i.e., MJ0170 (Fig. 1 *A*), which further revealed a remarkable similarity and phylogenetic relationship with its archaeal and bacterial homologs (Fig. 2, *A* and *B*). Such multiple repetition of the primary structural domains of the protein points to a duplication of the *mscL*-like progenitor gene followed by divergence similar to that proposed for the evolution of voltage-gated ion channels (Strong et al., 1993). Significantly, the conserved residues include those that were identified to be important for the MscL function. When mutated, several of those residues, such as G22 (G23

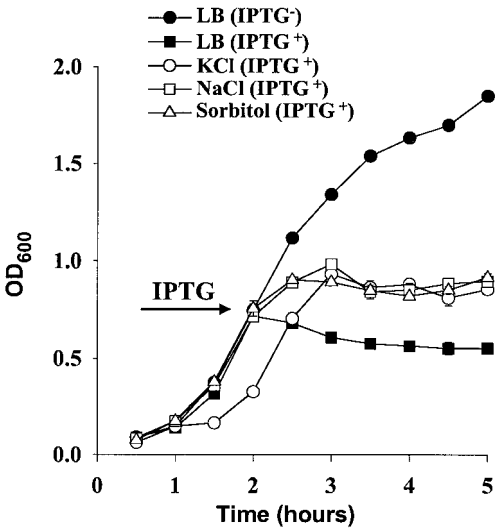



FIGURE 6 The effect of expression of MscMJ on *E. coli* growth. *E. coli* cells expressing *mscMJ* were growth inhibited. *mscMJ* gene expression was induced with IPTG once the cell culture reached mid-log phase $OD_{600}$ ~0.6, which corresponded to 2–2.5 h before addition of IPTG (marked with the *arrow*). The growth was partially rescued in media of high osmolarity containing either 300 mM NaCl, 300 mM KCl, or 600 mM sorbitol. ● and ■, growth curves of noninduced and induced-with-IPTG *E. coli* cells harboring pQE-32MJ0170 plasmid, respectively. Open symbols represent growth curves after induction with IPTG in media containing the following osmoprotectants: KCl (○), NaCl (□), and sorbitol (△).

of MscMJ), G26 (G27 of MscMJ), and K31 (K32 of MscMJ) (Fig. 1 *A*), produce bacterial phenotypes with impaired growth and increased MscL sensitivity to pressure in patch-clamp experiments (Blount et al., 1997; Ou et al., 1998). According to the three-dimensional crystal structure of MscL (Chang et al., 1998) these residues are located at or near the channel gate and thus seem to be important for the channel gating.

The lack of MscL homologs in the current archaeal databases and the presence of the MscL-like structural motif among MscMJ homologs representing three species belonging to two different phyla further suggest the origin of prokaryotic MS channels from a common ancestor, which most likely resembled MscL (Fig. 2, *A* and *B*). The common ancestry and evolutionary divergence is also reflected in the phylogenetic tree of aligned sequences, which are clustered into three main branches.

MscL (136 residues) is approximately two and half times smaller than MscS (286 residues) or its archaeal counterpart MscMJ (350 residues). The most diverged homologs, which occupy the third branch on the phylogenetic tree, are approximately three times larger than MscS or MscMJ homologs. Such gradual increase in the size of related proteins harboring the basic pattern, which can be traced back to the MscL sequence, further supports the common ancestry, gene duplication and divergence.

As discussed further, the common ancestral origin and the high level of conservation of the primary structure that MscMJ shares with MscL and MscS is largely matched by the similarity in functional properties of these prokaryotic MS channels.

## Mechanosensitivity of MscMJ

In terms of its mechanosensitivity MscMJ is functionally more closely related to MscS than MscL, as MscS (i.e.,YggB) and MscMJ require less pressure for activation than does MscL (Berrier et al., 1996). It can be shown that by multiplying $p_{1/2}$ and $\alpha$ one can obtain an estimate of the free energy of activation for a MS channel (see Materials and Methods):

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

Consequently, for MscMJ $\Delta G_o \approx 5kT$, which is approximately three times less than $\Delta G_o = 18.6kT$ estimated for MscL (Sukharev et al., 1999) but is similar to $\Delta Go = 7kT$ obtained for MscS (Martinac, unpublished; see Materials and Methods). It was previously shown that the negative pressure necessary to activate MscS is about two times smaller than the pressure required for activation of MscL in giant spheroplasts of *E. coli* (Blount et al., 1996; Berrier et al., 1996). Like MscS, MscMJ could also be activated by voltage, further indicating a close functional relationship to MscS (Martinac et al., 1987).

Lipid bilayers of cell membranes of Archaea consist of diphytanylglycerol-diether or -tetraether or both (Doolittle, 1999) and are therefore chemically very different from phospholipids of bacteria or eukaryotes, which are characterized by ester linkages between glycerol and acyl chains (Kates, 1993). However, in terms of physical properties relevant to gating of MS channels by mechanical force, lipids of *M. jannaschii* seem not to differ from phospholipids, because MscMJ exhibits mechanosensitivity in bilayers of azolectin liposomes.

## Conductance and selectivity of MscMJ

Using the value for the channel conductance $g = 270$ pS (Fig. 4, *B* and *C*) we estimated the size of the channel pore to be $d_{pore} \approx 9$ Å. The following expression was derived from Hille's model (Hille, 1968) to calculate the diameter of the channel pore:

$$d_{pore} = \frac{\rho g}{\pi}\left(\frac{\pi}{2} + \sqrt{\left(\frac{\pi}{2}\right)^2 + \frac{4\pi l}{\rho g}}\right), \quad (2)$$

where the resistivity of the recording solution $\rho$ was measured as 49.7 Ωcm and $l \approx 40$ Å was the bilayer thickness (Cruickshank et al., 1997). Thus, the pore of MscMJ is approximately three times smaller than that of the MscL pore, which was estimated to be ~35 Å (Cruickshank et al., 1997), and two times smaller than the size of the MscS pore, which from its conductance of ~1 nS (Sukharev et al.,

1993) was calculated to be ~18 Å. This result is consistent with the finding that sensitivity to applied pressure of bacterial MS channels correlates with their conductance (i.e., pore size) (Berrier et al., 1996).

The ionic selectivity of MscMJ is very different from any of the known prokaryotic MS channels. MscMJ exhibits a sixfold preference for cations over anions, whereas MscS has a threefold preference for anions over cations (Martinac et al., 1987) and MscL lacks ionic selectivity (Sukharev et al., 1993). Thus, in terms of its ionic preference, MscMJ resembles eukaryotic stretch-activated cationic (SA-CAT) MS channels (Hamill and McBride, 1996). The preference for cations and in particular for $Ca^{2+}$ exhibited by MscMJ (Table 1) might have evolved as a requirement for life in deep sea hydrothermal vents, a natural environment of *M. jannashii* (Bult et al., 1996).

### Physiological role of MscMJ

Expression of MscMJ affected the growth of host *E. coli* cells (Fig. 6). Although it is possible that expression of the MscMJ protein is toxic per se to *E. coli*, the impaired growth of bacterial cells might have also resulted from MscMJ being more frequently open in *E. coli* than in *M. jannashii.* If this were the case, it would lead to an increase in the leak of ions and/or vital cellular osmoprotectants out of the cell interior via MscMJ. The partial rescue of bacterial cells in media of higher osmolarity suggests that the level of cellular turgor needed to activate MscMJ, relative to the extracellular environment, may indeed be higher in *E. coli* than in the marine *M. jannashii*. Interestingly, all identified MscMJ (i.e., MJ0170) homologs have an asparagine (N182) in the third transmembrane helix at the position corresponding to the K31 residue of MscL (Fig. 2 *A*), which when mutated led to the impairment in growth of *E. coli* cells that could be partially rescued by high-osmolarity media (Ou et al., 1998). Alternatively, MscMJ may act as a bona fide calcium channel (Table 1) that allows calcium to leak into the bacterial host cells causing the calcium levels to reach toxic intracellular concentrations.

At first glance this may appear counterintuitive, however, taking into account the enormous pressures experienced by microorganisms living at the sea bottom; nevertheless, MscMJ and its homologs could in analogy to MscL and MscS also serve as a safety valve in osmoregulation of benthic archaeal or bacterial cells according to environmental cues. Although not much is known about turgor pressure in archaeal cells, cell turgor is essential for growth and cell wall synthesis in prokaryotic microbes, as stretch of the cellular envelope resulting from turgor is required for enlargement of the envelope and consequently for growth of bacterial cells (Csonka and Epstein, 1996). In addition, bacterial MS channels were shown to respond to sudden changes in environmental osmotic pressures (Ajouz et al., 1998; Levina et al., 1999). Such changes can also be expected to occur in the deep sea near hydrothermal chimneys, the natural habitat of *M. jannashii*.

## CONCLUSIONS

In this study we demonstrated that the hypothetical protein MJ0170 of the archaeon *M. jannashii*, which we rename MscMJ, is a mechanosensitive channel protein with properties very similar to bacterial MS channels, MscL and MscS. MscMJ is activated by mechanical force transmitted via the lipid bilayer alone, which is also consistent with our finding that amphipaths act as activators of this channel. Interestingly, the bilayer model also applies to eukaryotic MS channels (Zhang et al., 2000), which indicates that the bilayer mechanism is the oldest means of activating MS channels by mechanical force. The important implication of this result is that the mechanism underlying mechanosensitivity of MS channels may have first evolved in Bacteria and Archaea and then been conserved in eukaryotes. Though functionally behaving similar to MscS rather than MscL, the MscMJ channel contains stretches of amino acids with a high proportion of identical residues not only to MscS but also to the TM1 transmembrane domain of MscL. Because this important structural domain of bacterial MS channels is preserved within two putative transmembrane domains of MscMJ, which further shows a high degree of homology with not only archaeal but also bacterial MS channels, we propose that MscL, MscS, and MscMJ belong to the same family of prokaryotic MS channels evolved from a common MscL-like molecule via gene duplication and subsequent diversification of the progenitor gene.

We thank Dr. Ian Booth from the University of Aberdeen, Scotland, UK, for providing *E. coli* strain MJF465. Also, we thank Mr. J. Steer and Mr. Paul Chan for help with graphics, Dr. N. Butcher for advice on statistics, and Dr. A. Oakley for help with installation of sequence alignment programs, all at the University of Western Australia. Finally, we thank Dr. A. Ghazi, CNRS at the University of Paris-Sud, France; Dr. Owen P. Hamill from the University of Texas, Galveston, TX; and Dr. David Joyce and Mr. Jay Steer from the University of Western Australia for helpful discussion and critical reading of the manuscript.

This study was supported by the grant A09941004 from the Australian Research Council.

## REFERENCES

Ajouz, B., C. Berrier, M. Besnard, B. Martinac, and A. Ghazi. 2000. Contributions of the different extramembraneous domains of the mechanosensitive ion channel MscL to its response to membrane tension. *J. Biol. Chem.* 275:1015–1022.

Ajouz, B., C. Berrier, A. Garrigues, M. Besnard, and A. Ghazi. 1998. Release of thioredoxin via mechanosensitive channel MscL during osmotic downshock of *Escherichia coli* cells. *J. Biol. Chem.* 273: 26670–26674.

Barinaga, M. 1994. Molecular evolution: Archaea and eukaryotes grow closer. *Science.* 264:1251.

Batiza, A. F., I. Rayment, and C. Kung. 1999. Channel gate! Tension, leak and disclosure. *Struct. Fold. Design.* 7:R99–R103.

Berrier, C., M. Besnard, B. Ajouz, A. Coulombe, and A. Ghazi. 1996. Multiple mechanosensitive ion channels from *Escherichia coli*, activated at different thresholds of applied pressure. *J. Membr. Biol.* 151: 175–187.

Blount, P., M. Schroeder, and C. Kung. 1997. Mutations in a bacterial mechanosensitive channel change the cellular response to osmotic stress. *J. Biol. Chem.* 272:32150–32157.

Blount, P., S. I. Sukharev, P. C. Moe, B. Martinac, and C. Kung. 1999. Mechanosensitive channels in bacteria. *Methods Enzymol.* 294: 458–482.

Blount, P., S. I. Sukharev, M. J. Schroeder, S. K. Nagle, and C. Kung. 1996. Single residue substitutions that change gating properties of a mechanosensitive channel in *Escherichia coli. Proc. Natl. Acad. Sci. U.S.A.* 93:11652–11657.

Bult, C. J., O. White, G. J. Olsen, L. Zhou, R. D. Fleischmann, G. G. Sutton, J. A. Blake, L. M. FitzGerald, R. A. Clayton, J. D. Gocayne, A. R. Kerlavage, B. A. Dougherty, J. F. Tomb, M. D. Adams, C. I. Reich, R. Overbeek, E. f. Kirkness, K. G. Weinstock, J. M. Merrick, A. Glodek, J. L. Scott, N. S. M. Geoghagen, J. F. Weidman, J. L. Fuhrmann, D. Nguyen, T. R. Utterback, J. M. Kelley, J. D. Peterson, P. W. Sadow, M. C. Hanna, M. D. Cotton, K. M. Roberts, M. A. Hurst, B. P. Kaine, M. Borodovsky, H. P. Klenk, C. M. Fraser, H. O. Smith, C. R. Woese, and J. C. Venter. 1996. Complete genome sequence of the methanogenic Archaeon, *Methanococcus jannaschii*. *Science.* 273: 1058–1073.

Chang, G., R. Spencer, A. Lee, M. Barclay, and D. Rees. 1998. Structure of the MscL homologue from *Mycobacterium tuberculosis*: a gated mechanosensitive ion channel. *Science.* 282:2220–2226.

Cruickshank, C., R. Minchin, A. LeDain, and B. Martinac. 1997. Estimation of the pore size of the large-conductance mechanosensitive ion channel of *Escherichia coli. Biophys. J.* 73:1925–1931.

Csonka, L. N., and W. Epstein. 1996. Osmoregulation. *In Escherichia coli* and *Salmonella:* Cellular and Molecular Biology, 2nd ed. F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K. Brooks Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger, editors. ASM Press, Washington D.C.1210–1225.

Delcour, A. H., B. Martinac, J. Adler, and C. Kung. 1989. Modified reconstitution method used in patch-clamp studies of *Escherichia coli* ion channels. *Biophys. J.* 56:631–636.

Doolittle, W. F. 1999. Phylogenetic classification and the universal tree. *Science.* 284:2124–2128.

Garcia-Añovernos, J., and D. P. Corey. 1997. The molecules of mechanosensation. *Annu. Rev. Neurosci.* 20:567–594.

Hamill, O. P., A. Marty, E. Neher, B. Sackmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch. Eur. J. Physiol.* 391:85–100.

Hamill, O. P., and D. W. McBride, Jr. 1996. The pharmacology of mechanogated membrane ion channels. *Pharmacol. Rev.* 48:231–252.

Häse, C. C., A. C. LeDain, and B. Martinac. 1995. Purification and functional reconstitution of the recombinant large mechanosensitive ion channel (MscL) of *Escherichia coli. J. Biol. Chem.* 270:18329–18334.

Häse, C. C., A. C. LeDain, and B. Martinac. 1997. Molecular dissection of the large mechanosensitive ion channel (MscL) of *Escherichia coli*: mutants with altered channel gating and pressure sensitivity. *J. Membr. Biol.* 157:17–25.

Hille, B. 1968. Pharmacological modification of the sodium channels of frog nerve. *J. Gen. Physiol.* 51:99–219.

Hille, B. 1992. Ionic Channels of Excitable Membranes, 2nd ed. Sinauer, Sunderland, MA.

Howard, J., W. M. Roberts, and A. J. Hudspeth. 1988. Mechanoelectrical transduction by hair cells. *Annu. Rev. Biophys. Chem.* 17:99–124.

Kates, M. 1993. Membrane lipids of Archaea. *In* The Biochemistry of Archaea *(Archaeobacteria).* M. Kates, D.J. Kushner, and A.T. Matheson, editors. Elsevier, London. 261–295

Le Dain, A. C., N. Saint, A. Kloda, A. Ghazi, and B. Martinac. 1998. Mechanosensitive ion channels of the archaeon *Haloferax volcanii. J. Biol. Chem.* 273:12116–12119.

Levina, N., S. Totemeyer, N. R. Stokes, P. Louis, M. A. Jones, and I. R. Booth. 1999. Protection of *Escherichia coli* cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. *EMBO J.* 18:1730–1737.

Martinac, B. 1993. Mechanosensitive ion channels: biophysics and physiology. *In* Thermodynamics of Membrane Receptors and Channels. M.B. Jackson, editor. CRC Press, Boca Raton, FL. 327–351.

Martinac, B. 1999. Mechanosensitive ion channels: universal biological transducers of mechanical stimuli. *Austr. Biochem.* 8(3):6–10.

Martinac, B., J. Adler, and C. Kung. 1990. Mechanosensitive ion channels of *E. coli* activated by amphipaths. *Nature.* 348:261–263.

Martinac, B., M. Buechner, A. H. Delcour, J. Adler, and C. Kung. 1987. Pressure-sensitive ion channel in *Escherichia coli. Proc. Natl. Acad. Sci. U.S.A.* 84:2297–2301.

Martinac, B., A. H. Delcour, M. Buechner, J. Adler, and C. Kung. 1992. Mechanosensitive ion channels in bacteria. *In* Comparative Aspects of Mechanoreceptor Systems. F. Ito, editor. Springer Verlag, Berlin. 3–18.

Moe, P. C., P. Blount, and C. Kung. 1998. Functional and structural conservation in the mechanosensitive channel MscL implicates elements crucial for mechanosensation. *Mol. Microbiol.* 28:583–592.

Morell, V. 1996. Life's last domain. *Science.* 273:1043–1045.

Oakley, A., B. Martinac, and M. Wilce. 1999. Structure and function of the bacterial mechanosensitive channel of large conductance. *Protein Sci.* 8:1915–1921.

Ou, X., P. Blount, R. J. Hoffman, and C. Kung. 1998. One face of a transmembrane helix is crucial for mechanosensitive channel gating. *Proc. Natl. Acad. Sci. U.S.A.* 95:11471–11475.

Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. *Science.* 276:734–740.

Patel, A., E. Honoré, F. Maingret, F. Lesage, M. Fink, F. Duprat, and M. Lazdunski. 1998. A mammalian two pore domain mechano-gated S-like $K^+$ channel. *EMBO J.* 17:4283–4290.

Reithmeier, R. A. F., and C. M. Deber. 1992. Intrinsic membrane protein structure: principle and prediction. *In* The Structure of Biological Membranes. P. Yeagle, editor. CRC Press, Boca Raton, FL. 337–393.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Spencer, R. H., G. Chang, and D. C. Rees. 1999. Feeling the pressure: structural insights into a gated mechanosensitive channel. *Curr. Opin. Struct. Biol.* 9:448–454.

Stein, J. L., and M. I. Simon. 1996. Archaeal ubiqity. *Proc. Natl. Acad. Sci. U.S.A.* 93:6228–6230.

Strong, M., K. G. Chandy, and G. A. Gutman. 1993. Molecular evolution of voltage sensitive ion channel genes: on the origins of electrical excitability. *Mol. Biol. Evol.* 10:221–242.

Sukharev, S. I., P. Blount, B. Martinac, F. R. Blattner, and C. Kung. 1994. A large mechanosensitive channel in *E. coli* encoded by *mscL* alone. *Nature.* 268:265–268.

Sukharev, S. I., P. Blount, B. Martinac, and C. Kung. 1997. Mechanosensitive channels of *Escherichia coli*: the MscL gene, protein, and activities. *Annu. Rev. Physiol.* 59:633–657.

Sukharev, S. I., B. Martinac, V. Y. Arshavsky, and C. Kung. 1993. Two types of mechanosenitive channels in the *E. coli* cell envelope: solubilization and functional reconstitution. *Biophys. J.* 65:177–183.

Sukharev, S. I., W. J. Sigurdson, C. Kung, and F. Sachs. 1999. Energetic and spatial parameters for gating of the bacterial large conductance mechanosensitive channel, MscL. *J. Gen. Physiol.* 113:525–539.

Voorhorst, W. G., A. Warner, V. M. de Vos, and R. J. Siezen. 1997. Homology modelling of two subtilisin-like serine proteases from the hyperthermophilic archaea *Pyrococcus furiosus* and *Thermococcus stetteri. Protein Eng.* 10:905–914.

Woese, C. R. 1994. There must be a prokaryote somewhere: microbiology's search for itself. *Microbiol. Rev.* 58:1–9.

Zhang, Y., F. Gao, V. L. Popov, J. Wen, and O. P. Hamill. 2000. On the discrepancy between whole cell and membrane patch mechanosensitivity of *Xenopus* oocytes. *J. Physiol.* 523:117–130.

Zoratti, M., and A. Ghazi. 1993. Stretch activated channels in prokaryotes. *In* Alkali Transport Systems in Prokaryotes. E.P. Bakker, editor. pp. CRC Press, Boca Raton, FL. 349–358.