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A mechanosensitive ion channel in *Schizosaccharomyces pombe*

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Protoplast protuberances (blebs) of *Schizosaccharomyces pombe* were examined using the patch-clamp technique. In addition to several voltage-gated ion channels, we encountered the activities of a mechanosensitive ion channel with a conductance of 180 pS. Microscopic currents of one or two units were observed in some excised patches and ensemble currents of several tens of units were observed in all blebs examined in whole-bleb configuration. This channel opens at pressures of cm Hg applied to whole blebs and it passes cations, including Ca^{2+}. It is inactivated by membrane depolarizations and blocked by Gd^{3+}. We discuss the possible functions of such a channel, including its activation upon cell cycle dependent cytoskeletal reorganizations.

Key words: Ca^{2+}/cell cycle/cell shape/mechanosensitive channel/*S.pombe*

Introduction

The fission yeast, *Schizosaccharomyces pombe*, has been developed into a powerful experimental system, especially for the genetic and molecular analysis of cell cycle controls (Nurse, 1991a,b). The cytology, intermediary metabolism, transmission genetics and molecular biology of this organism have now been greatly advanced (Nasim *et al.*, 1989; Moreno *et al.*, 1990). Although the plasma membrane H^+-ATPase and its corresponding gene have been studied in detail (Ghislain *et al.*, 1988; Goffeau *et al.*, 1989), the electric and ionic aspects of the physiology of fission yeast, like those of most microbial organisms, have been largely unexplored. This report begins a series of studies of the ion channels of *S.pombe*, using the patch-clamp technique.

The patch-clamp technique (Hamill *et al.*, 1981) has not only made possible the recording of the activities from individual ion channels, but also relaxed the requirement for large cell size in electrophysiological studies. Thus, we now witness a rapid growth in knowledge of ion channels from small animal cells, plant cells (Hedrich and Schroeder, 1989) and even microbes (Saimi *et al.*, 1988, 1992; Kung *et al.*, 1990). Among the microbes examined with the patch-clamp technique are the ciliated protozoan *Paramecium tetraurelia* (Kubalski *et al.*, 1989; Saimi and Ling, 1990), the Gram-negative bacterium, *Escherichia coli* (Martinac *et al.*, 1987, 1990; Delcour *et al.*, 1989), the budding yeast, *Saccharomyces cerevisiae* (Gustin *et al.*, 1986, 1988), the bean-rust fungus, *Uromyces appendiculatus* (Zhou *et al.*, 1991) and now the fission yeast, *S.pombe*.

Ion channels are described as being gated, i.e. the probability of them being in the open conformation is regulated. The best known channels, especially those in the excitable membranes of nerves and muscles, are gated by the transmembrane voltage (Hille, 1984). Voltage-gated channels have also been found in microbes (Kung and Saimi, 1985; Gustin *et al.*, 1986; Delcour *et al.*, 1989). However, many other channels are gated by other principles, such as external ligands, internal second messengers, through G-proteins or calmodulin. A class of mechanosensitive (MS) ion channels that are gated by stretch forces on membranes was first discovered by Guharay and Sachs (1984) and Brehm *et al.* (1984) in muscle cells. Such channels have now also been found in heart cells, neurons, oocytes, endothelial cells, kidney cells and plant cells (for a review see Sachs, 1988). Interestingly, MS channels that are activated by small membrane tensions of physiological range have also been discovered in *E.coli* (Martinac *et al.*, 1987, 1990) and in three fungal cells: *S.cerevisiae* (Gustin *et al.*, 1988), *U.appendiculatus* (Zhou *et al.*, 1991) and *S.pombe* (this report). Whole-cell ensemble MS currents as well as unitary currents can readily be measured in these microbes. The MS channels in these microbes clearly differ in their conductances, ion selectivities and voltage regulations.

Many functions, such as touch, hearing, proprioception and stomatal regulation, for instance, have been suggested for MS channels (Sachs, 1988). Germ tubes from spores of *U.appendiculatus* exhibit an exquisite sense for the topography of bean leaves in search of stomata, through which they invade the leaves. Chemically inert ridges, 0.45-0.70 μm in height, about the height of the stomatal lips, induce the differentiation of the germ tubes into invasive structures (Hoch *et al.*, 1987). The MS channel recently characterized in the germ tube spheroplasts of *U.appendiculatus* could be the topographic sensor (Zhou *et al.*, 1991). The MS channels in free-living bacteria and yeasts may have other less obvious functions. They may be used in osmoregulation. Being responsive to membrane tension, the MS channels gauge the cytoplasmic turgor and can thereby measure sudden changes in water content of the environment (Gustin *et al.*, 1988; Kung *et al.*, 1990). Alternatively, or in addition, they may participate in cell cycle events, since the channel-gating tension is resisted by the cytoskeleton (Sachs, 1988; Martinac *et al.*, 1992), which undergoes drastic reorganizations at different stages in the cell cycle (Marks and Hyams, 1985; Hagan and Hyams, 1988). *S.pombe* is free-living and is amenable to modern genetic and molecular biological research. It has MS channels of a larger conductance and a higher apparent mecahnosensitivity than those of *S.cerevisiae* (see below). Furthermore, its cell cycle closely resembles that of metazoa. It therefore seems important to examine how this yeast regulates its membrane potential and its internal concentrations of ions, especially Ca^{2+}, so as to provide a foundation for future studies in the cell physiology of this organism.

Results

The blebs: protoplast protuberances

The gigaohm seals required for recording unitary current could not be formed between the recording pipette and the cell wall of *S.pombe*. However, they could readily be formed between the tip of the patch-clamp pipette and the exposed plasma membrane of blebs referred to as 'protoplast protuberances' by Kobori *et al.* (1989) and Vondrejz *et al.* (1990). Seals of 10–50 GΩ resistance were often formed instantly and lasted for hours. On-bleb mode was usually established first, which could be converted to the excised inside-out patch mode by rapid perfusion. Most commonly, we sustained an on-cell suction to break the patch for tens of seconds, thereby establishing the whole-bleb recording mode, which could also be converted to excised, outside-out patch mode. Although occasionally detached, these blebs most often still adhered to the walled rod-shaped cells, from which they emerged (Figure 1). Cells were ~3 μm in diameter and 6–15 μm in length and the spherical blebs were ~2–5 μm in diameter. Thus the bleb surface accounted for about a quarter to a half of the total surface of an *S.pombe* cell.

Although we focus on the MS channels in this report, we have also observed the activities of at least three other channel types that appeared to be voltage-activated and not mechanosensitive. They had unit conductances of ~15 pS, 30 pS, 250 pS and 400 pS; the last two types came with multiple substates and may reflect the same type of channel (data not shown). The MS channels of *S.pombe* could readily be activated by pressures of cm Hg exerted into the bleb through the pipette. Pressure of 1 cm Hg is equivalent to the osmotic pressure generated by a difference of only 0.54 mOsM concentration across a membrane. The report below is based on a study of >30 patches and >200 blebs. The MS activities of one or two units were observed in ~1/3 of the patches. Ensemble MS currents of several tens of units were observed in every whole-bleb preparation. Data presented are mostly from whole-bleb records.

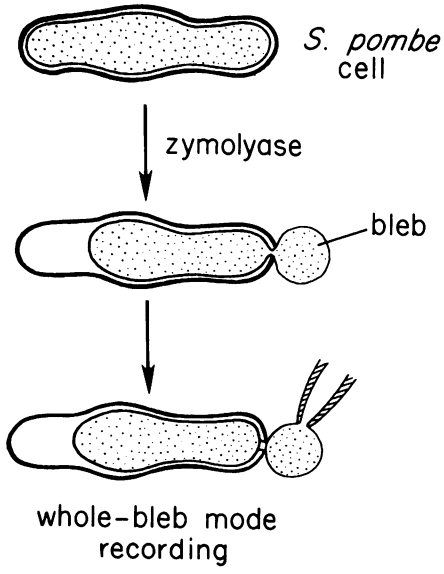

Fig. 1. A diagram of the method for producing a protoplast protuberance (bleb) from an *S.pombe* cell, partly based on the observations of Vondrejz *et al.* (1990).

Channels activated by pressure

Seal resistances were not altered by the application of pressure into the blebs. Figure 2A shows that without applied pressure there was little activity of the MS channels recorded from a bleb. A small pressure (2.0 cm Hg, 1 cm Hg = 1330 Pa) activated some of these channels (Figure 2A). The activities reflecting the openings and closings of individual units can be resolved better at a higher time resolution (Figure 2A, bottom two traces). The unitary current corresponded to a conductance of 180 pS (in a symmetric 180 mM K^+ solution, see below). The discrete conductance was also evident in current amplitude histograms such as Figure 2B. These MS channels could also be activated by either pressure or suction on excised patches (data not shown) although, as expected, forces larger than those for whole blebs were required.

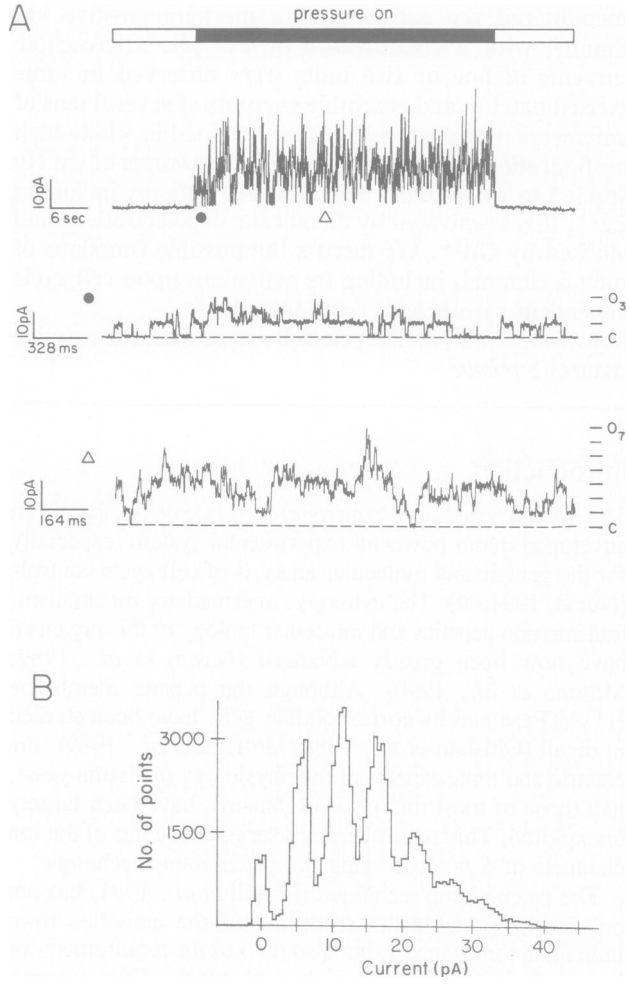

Fig. 2. The activities of MS channels in *S.pombe*. Recording was performed in the whole-bleb mode. (**A**) A sample trace of channel activities upon the application of a pressure of 2.0 cm Hg to a protoplast protuberance (bleb). The membrane was held at +30 mV. Two portions of the activities are expanded at a higher time resolution as the bottom traces. They illustrate lower or higher activities of the same ensemble of MS channels in a bleb. (**B**) Amplitude histogram showing the currents recorded from this bleb for 55 s under 2.0 cm Hg pressure. Note that the amplitude peaks for closed, one-open, two-open, for example, are equally spaced. Pipette solution: (in mM) 180 KCl, 0.01 $CaCl_2$, 5 HEPES, adjusted to pH 7.2 with KOH; bath solution: (in mM) 100 NaCl, 20 KCl, 50 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, adjusted to pH 7.2 with KOH.

Pressure dependence

The sensitivity of the MS channel to pressure was quantified. Figure 3A shows that the channels in a bleb became progressively more active as applied pressure was increased from 0 to 10.0 cm Hg. Figure 3B plots the results from this bleb, which was ~2 μm in diameter (right curve) and another larger bleb of ~4 μm in diameter (left curve). Here, NP_o is plotted against the applied pressure. N is defined as the apparent number of identical channels, P_o is the single-channel open probability. NP_o is calculated with the aid of a computer from I/i where I is the ensemble current averaged over 20 s and i is the single-channel current. In the case shown in Figure 3A and B, right curve, we estimated that >40 channels were activated. In the case shown in Figure 3B, left curve, >80 channels were activated. Different blebs had exponentially rising curves that did not coincide on the pressure axis. MS channels on larger blebs were activated at lower pressures than those on smaller blebs, as dictated by Laplace's law. When the open probability of MS channel molecules per unit area, DP_o, is plotted as a function of tension (calculated from the applied pressure) curves from the two blebs coincide (Figure 3C). This conincidence supports the view that MS channels are activated by membrane tension and not directly by pressure (Gustin *et al.*, 1988). As with spheroplasts of *S.cerevisiae*, although we could activate all the MS channels with large pressures in rare cases (Gustin, 1991), typical blebs could not sustain those pressures.

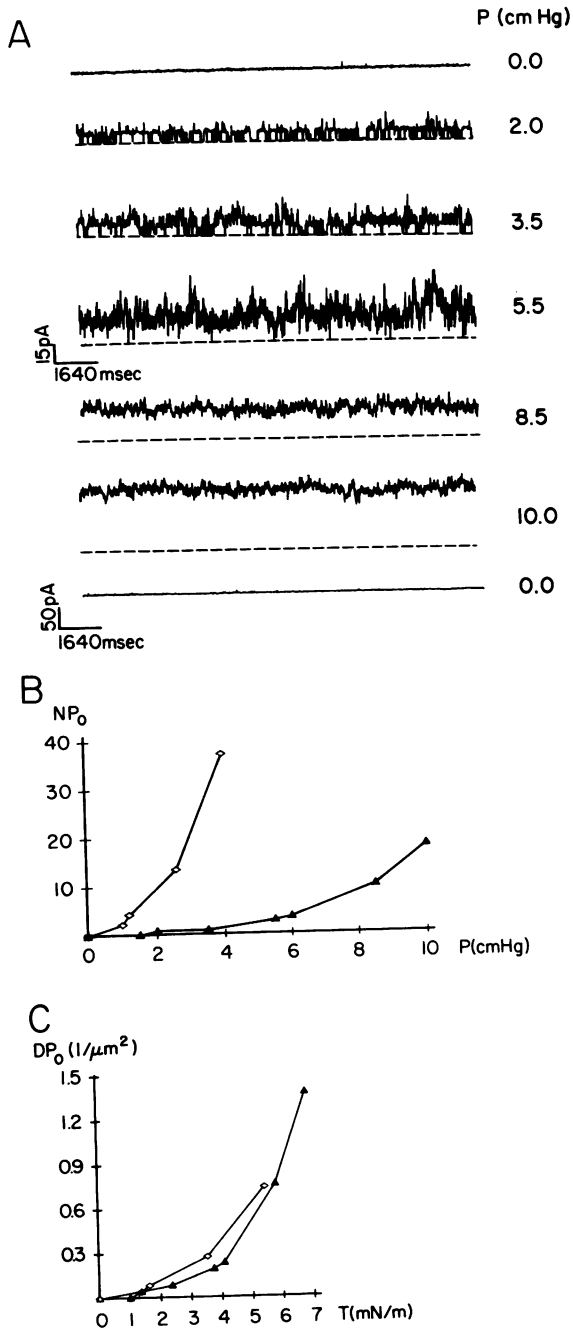

Fig. 3. Pressure sensitivity of the MS channels recorded in whole-bleb mode. (**A**) Traces from a bleb under different pressures marked on the right, note the scale change. The 15 pA scale applies to the upper four traces; the 50 pA scale to the lower three. (**B**) Shows the relationship between the total channel open probability (NP_o) and the applied pressure in cm Hg. Data from two blebs are plotted. Curve on the right from the same bleb shown in A. (**C**) Represents a replot of the data from B in forms of the open probability of channels per unit area (DP_o) against tension in mN/m, calculated from the applied pressure, showing that MS channel molecules from different blebs respond to the membrane tension in the same manner. The membrane voltage was +40 mV; solutions as in Figure 2.

MS channel passes cations

The plot of the unitary current (I) against voltage (V) of the MS channel activities recorded in a symmetric 180 mM KCl solution has a slope conductance of 182.5 ± 8.4 pS (mean ± SD, n = 6). To test the channel's ion preference or selectivity, we first established the I/V relationship in the symmetric KCl solution (Figure 4A, triangles, see legend for solution compositions) before diluting the bath KCl to 1/5 through equimolar substitution of sorbitol to maintain the osmotic pressure (Figure 4A, diamonds). This dilution shifted the I/V curve to the left by 20.0 ± 5.1 mV (n = 3). Thus the MS channel clearly prefers K^+ over Cl^-, with a P_{K^+} / P_{Cl^-} of 3.6, calculated from the reversal potential.

The abilities of different monovalent cations to permeate this channel were compared. An I/V plot was first established in symmetric 180 mM CsCl solutions in both the bath and the pipette, and then the bath solution was replaced with equimolar KCl, RbCl or NaCl. The reversal potentials only slightly deviated from 0 mV in these replacements (Figure 4B). These results indicate a poor discrimination among the monovalent cations, although a preference series of $K^+ > Cs^+ \geq Rb^+ > Na^+$ could be discerned.

Because of the importance of Ca^{2+} in biological systems, the possibility that this divalent cation may permeate the MS channel was investigated. Beginning with the symmetric KCl solution and then substituting the bath with a $CaCl_2$ solution, the I−V plot shows only a slight leftward shift (Figure 4D). The reversal potential shows that Ca^{2+} permeates this MS channel with a permeability ratio of $P_{Ca^{2+}}/P_{K^+}$ = 0.46. Figure 3C gives sample traces from this experiment, showing outward K^+ currents (upper two traces) and inward Ca^{2+} currents (lower two traces).

Voltage sensitivity

We found that the MS channels became less active when membrane potential was positive (depolarization). Figure 5A shows that the channels in one bleb seldom visited the open state at positive voltages, even though the bleb was under

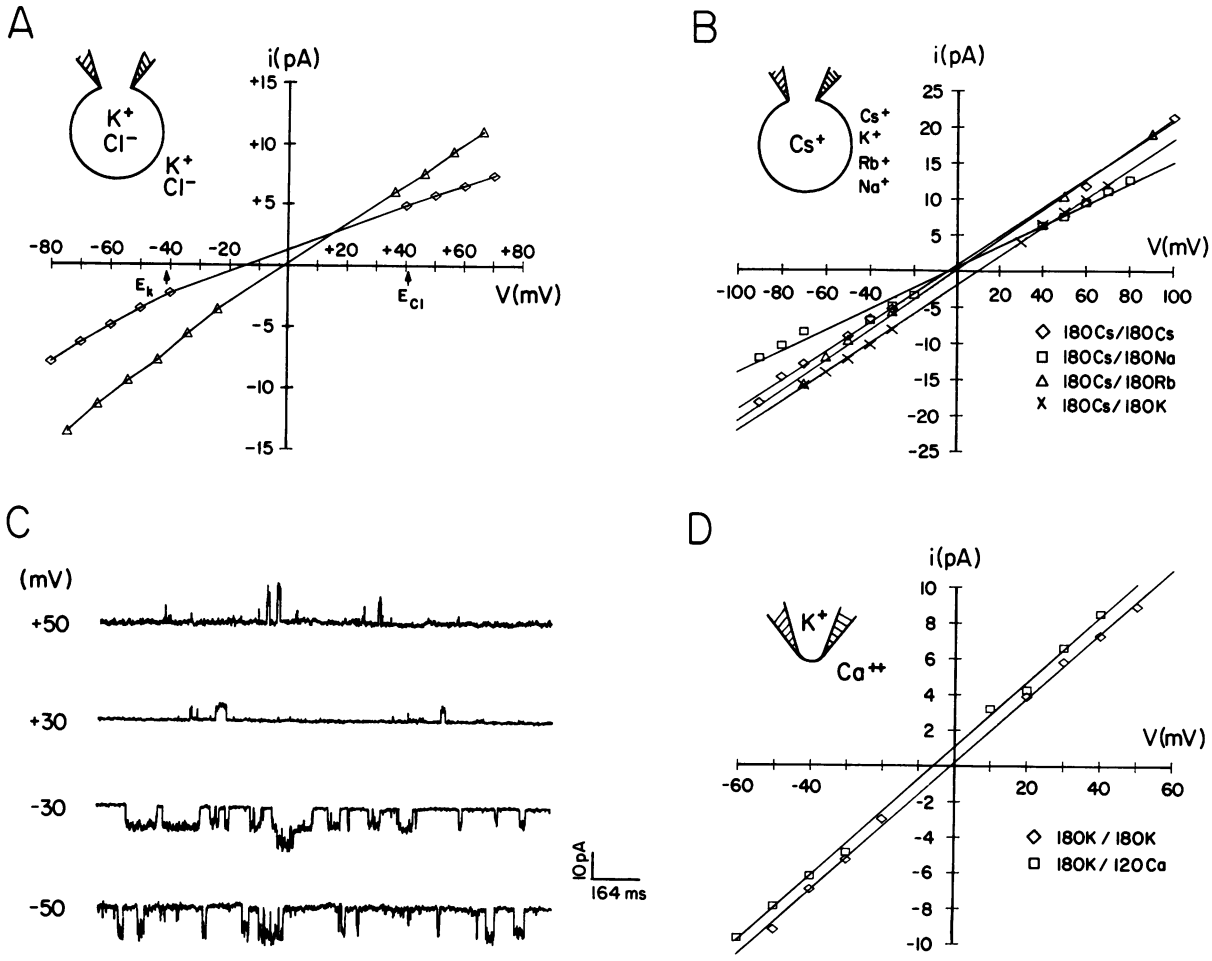

Fig. 4. Tests of ion selectivity of the MS channel. (**A**) Current–voltage (I–V) plots of the MS channels activated in whole-bleb mode at 1.5 cm Hg pressure. Triangles: pipette solution and bath solution both contained (in mM) 180 KCl, 0.01 $CaCl_2$, 5 HEPES, adjusted to pH 7.2 with KOH. After the I–V relationship was established in this symmetric solution, the bath was replaced with (in mM) 36 KCl, 288 sorbitol, 0.01 $CaCl_2$, 5 HEPES, adjusted to pH 7.2 with KOH to establish a second I–V plot (diamonds). E_K and E_{Cl} mark the calculated equilibrium potentials of K^+ and Cl^- in the asymmetric condition. (**B**) I–V plots from a different bleb. The pipette solution contained (in mM) 180 CsCl, 0.01 $CaCl_2$, 5 HEPES, adjusted to pH 7.2 with CsOH. The starting bath solution was the same CsCl solution (diamonds). The bath was then sequentially changed to (in mM) 180 KCl (crosses), 180 NaCl (squares) and 180 RbCl (triangles), each with 0.01 $CaCl_2$, 5 HEPES, adjusted to pH 7.2 with KOH, NaOH or RbOH. (**C**) Sample traces from an excised outside-out patch under 2.0 cm Hg pressure, recorded with a pipette solution of 180 KCl and a bath solution of 120 $CaCl_2$, both with 5 HEPES, pH 7.2 with KOH or $Ca(OH)_2$. Note that both outward currents carried mainly by K^+ (upper traces) and inward currents carried mainly by Ca^{2+} (lower traces) can be driven across the bleb membrane by different voltages. (**D**) I–V plot from 180 KCl symmetric condition (diamonds) and the condition of C (squares).

a constant pressure of 2.0 cm (top three traces). These relatively inactive channels could be activated by additional pressure. Activity increased with membrane polarization steeply up to about −40 mV (traces 4, 5 and 6). At deeper negative potentials (Figure 5A, bottom trace, 5B broken line), the activities of the channel were difficult to assess since voltage-induced time-dependent adaptation became evident (below). The voltage regulation of MS channels was quantified and is shown in Figure 5B. It is evident that there is a steep voltage dependence between −40 and 0 mV. Voltage alone does not seem to gate the MS channels. Step voltage changes in blebs not under pressure did not activate these MS channels (data not shown).

Adaptation by negative potential

When the bleb membrane was stepped from a positive to a negative level, being under a constant pressure the MS channels quickly activated and then slowly declined over several seconds (Figure 6, top). This decline appears to be a form of adaptation; additional pressures could still activate them after their activity declines (data not shown). Stepping the potential from a negative to a positive level reduced the open probability as expected (Figure 5B) but did not cause any observable time-dependent decline (Figure 6, bottom).

Gd^{3+} blockage

The effects of Gd^{3+} on this channel were tested, since it blocks the MS channels in other organisms (Gustin *et al.*, 1988; Yang and Sachs, 1989; Zhou *et al.*, 1991). This lanthanide indeed blocked the MS channel of *S.pombe* at submillimolar concentrations (Figure 7A). The major effect of Gd^{3+} was on the open probability of the channel. The dose–response curve (Figure 7B) shows that the half effective concentration was ~500 μM. Gd^{3+} also slightly reduced the unitary conductance of the MS channel in a concentration-dependent manner, but did not abolish

conduction even at high concentrations (Figure 7C), suggesting a blockage mechanism similar to that in oocytes (Yang and Sachs, 1989).

Discussion

The currents observed when pressure is applied on the protoplast membrane of *S.pombe* are through ion channels and not through non-specific leaks in the membrane or at the juncture between the membrane and the pipette. First, the currents consist of units of a discrete size, dictated by a fixed 180 pS conductance (Figures 2–5). Artefactual leakage currents are not expected to be so regular. Secondly, unlike growth cones and cell bodies of snail neurons, where whole-bleb currents could not be found (Morris and Horn, 1991), *S.pombe*, like other fungal preparations, readily exhibits ensemble currents in whole-bleb mode (Figure 3). In fact, most of the currents analysed and presented here are recorded in whole-bleb mode and induced by small pressures. Thirdly, the pressures that half activate the channels in blebs of different sizes are in the range of a few centimeters Hg (Figure 3), similar to the pressures known to activate MS channels in other species. These are forces within physiological range; 1 cm Hg pressure is equivalent to the osmotic pressure induced by the difference of 0.54 mOsM solutes across the membrane. Fourthly, this MS channel much prefers cations to anions, unlike any non-specific leak (Figure 4A). Fifthly, the open probability of this MS channel is regulated by membrane voltage, both in activation and in adaptation (Figures 5 and 6). Sixthly, unlike leakage current, the MS current is reversibly blocked by submillimolar concentrations of Gd^{3+} (Figure 7), an agent known to block currents through other MS channels (Gustin *et al.*, 1988; Yang and Sachs, 1989; Zhou *et al.*, 1991). Although it seems clear that the mechanically-induced currents are through specific ion channels and not artefacts, the possibility that these channels are also gated or regulated by ligands or membrane proteins cannot be excluded by the present study. Voltage clearly regulates this MS channel (Figures 5 and 6). However, without an applied membrane tension, voltage between +100 and −100 mV alone does not activate the channel (X.-L.Zhou and C.Kung, unpublished results).

The MS channel in *S.cerevisiae* has a conductance of 36 pS (Gustin *et al.*, 1988). The MS channel studied here in *S.pombe*, however, has a much higher conductance of 180 pS. We also found a channel of a still higher conductance (600 pS) in the germ tube protoplast of

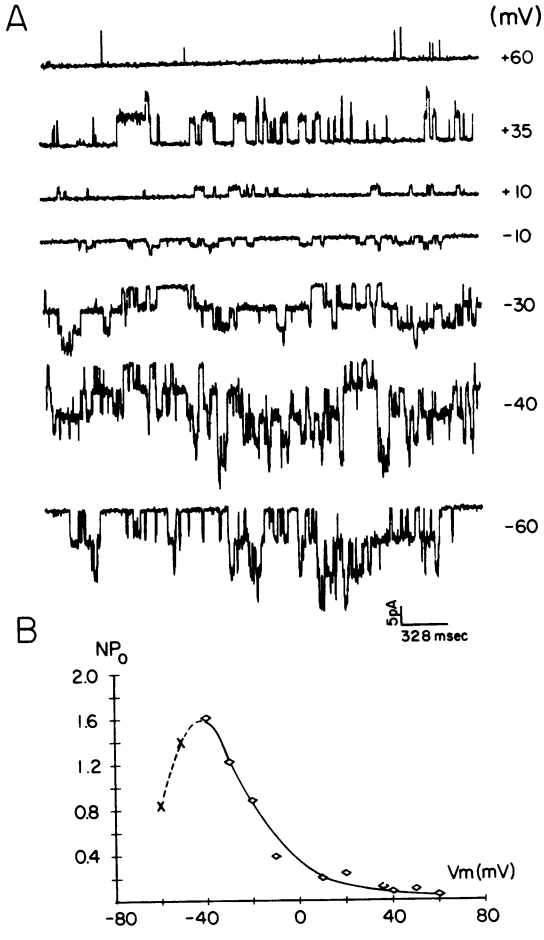

Fig. 5. Voltage regulation of the MS channels recorded from whole blebs. (**A**) Sample traces from a bleb under constant 2.0 cm Hg pressure. (**B**) Shows the open probability at different voltages from the same bleb. The broken line between the two points at −40 and −60 mV from the same bleb are from recording after the initial time-dependent adaptation. See text and Figure 6, top trace. The results are typical of >30 blebs studied and of three blebs quantitatively analysed. See Figure 2 for solutions.

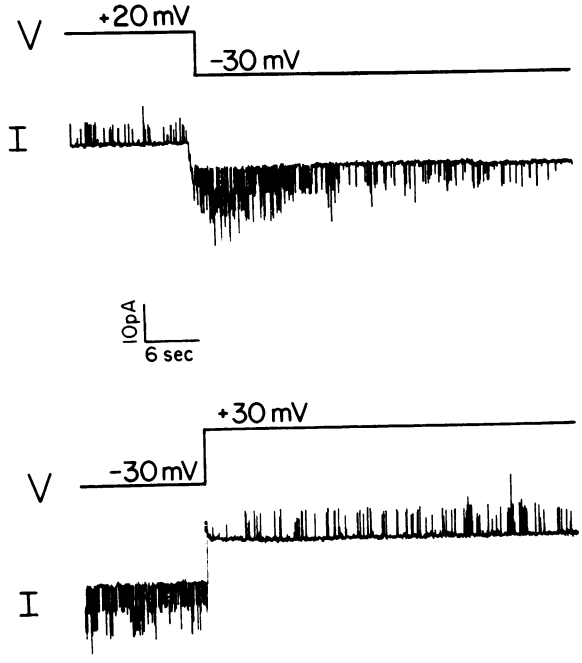

Fig. 6. Depolarization-induced adaptation of the MS channel. Activities were recorded in the whole-bleb mode at a sustained pressure of 2.0 cm Hg. Bleb membrane was first held at +20 mV and then stepped to −30 mV as shown at the top trace. The activities of the MS channel rapidly increased and then relaxed over 10 s (lower trace, upper panel). An inverse voltage-stepping protocol from −30 to +30 mV was tested after additional pressure (2.0 cm Hg) was applied to activate the MS channels. This hyperpolarization did not show the activity adaptation of the channels (lower panel). Pipette and bath solutions both contained (in mM): 180 KCl, 0.1 EGTA, 5 HEPES, adjusted to pH 7.2 with KOH.

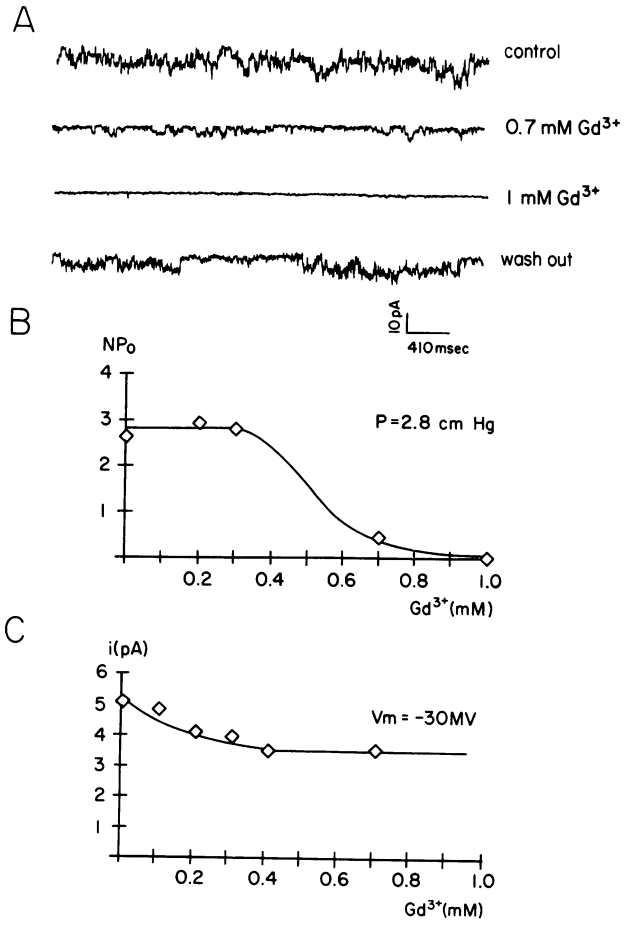

Fig. 7. Gd^{3+} blockage of the MS channel activities in whole-bleb mode. (**A**) Shows the current traces from a bleb in the absence and presence of different concentrations of Gd^{3+}. Typical of three blebs. (**B**) The open probability plotted against the concentration of Gd^{3+}. (**C**) Shows the unitary conductance of this channel in different concentrations of Gd^{3+}. See Figure 2 for solution compositions.

U.appendiculatus (Zhou *et al.*, 1991). Contrary to intuition, the higher conductance MS channels are more ion selective; P_{K^+}/P_{Cl^-} is 19.3 for *U.appendiculatus*, 3.6 for the *S.pombe* channel; and 1.6 for the *S.cerevisiae* channel. The kinetic properties of the MS channels from different species are also different. For example, the open channel current of *S.pombe* flickered rapidly (Figures 2–5), resulting in amplitude histograms (Figure 2B) with peaks broader than those we have seen in *U.appendiculatus* (Zhou *et al.*, 1991). The mechanical properties of the protoplasts from different fungal cells are different. Protoplasts of *U.appendiculatus* can sustain large pressures and can therefore readily reveal the saturation of whole-cell MS current, i.e. can exhibit the opening of all the MS channels of the cell (Zhou *et al.*, 1991). Those of *S.pombe* or *S.cerevisiae*, however, seldom allow saturation before the protoplasts break. It seems probable that the elastic elements parallel to the membrane, possibly cytoskeletons, differ in different kinds of cells. The MS channels of *S.pombe* are more easily activated by applied stretch force than those of *S.cerevisiae*. At a tension of 5 mN/m, *S.cerevisiae* shows a DP_o of $0.08/\mu m^2$ (Gustin *et al.*, 1988), while *S.pombe* shows a DP_o of $0.6/\mu m^2$ (Figure 3C). This shows that in *S.pombe* the MS channel protein is more sensitive and/or the membrane–cytoskeletal complex is more effective in transducing the tension to the channel.

Another difference among the MS channels from these three fungal species is their regulation by voltage. The *S.cerevisiae* mechanosensitive channel could become more active upon membrane depolarizations and show cooperativity of two conductance units (M.C.Gustin, X.-L.Zhou and C.Kung, unpublished results). The MS channel of *U.appendiculatus* behaves similarly to that of *S.cerevisiae* (Zhou *et al.*, 1991). The *S.pombe* channel, on the other hand, is less active upon depolarization (Figures 5 and 6). Depolarization inactivation of the *S.pombe* MS channel could be a part of a negative feedback loop to keep the time that the channel is open to a minimum *in vivo*: a stretch force causes the MS channel to open, permeation of both cations and anions results in a depolarization, and depolarization inactivates the channel. This negative feedback and the adaptation to hyperpolarization (Figure 6) seem to safeguard any prolonged opening of these MS channels at any membrane potentials *in vivo*.

The functions of the MS channels in fungal cells are unknown. Germ tubes from spores of *U.appendiculatus* exhibit topographic sensing (Hoch *et al.*, 1987). It is therefore tempting to think that the MS channel may serve as a topographic sensor, especially because Gd^{3+} blocks both the MS channel *in vitro* and the topographic sensory transduction *in vivo* (Zhou *et al.*, 1991). For the free-living fungi, one possible function of the MS channels is in the turgor detection and response. *S.cerevisiae*, for example, shows clear physiological responses to osmotic stress (Brown *et al.*, 1986; Blomberg *et al.*, 1988; Blomberg and Adler, 1989), although the sensor for the osmotic stress is obscure. Dilution of bath solution could indeed open the MS channels of *S.cerevisiae* (S.Loukin, X.-L.Zhou and C.Kung, in preparation).

Another possible role for MS channels is in the passage through the cell cycle. The mechanical properties of the cell are apparently not kept constant. The interphase cytoskeleton is dismantled during cell division: both microfilaments (Marks and Hyams, 1985) and microtubules (Hagan and Hyams, 1988) are grossly rearranged. MS channels respond to membrane tension (Martinac *et al.*, 1990) and this tension is counteracted by parallel resistive elements, most likely the submembrane cytoskeleton. Guharay and Sachs (1984) showed that cytochalasin B, which could disrupt the cytoskeleton, caused the MS channels of chick myoblasts to become far more sensitive to membrane tension. It is therefore probable that the MS channels are subjected to different membrane tensions as the cytoskeleton disassembles and reassembles during different stages in the cell cycle of many cell types, including yeasts. In early fish embryogenesis, MS channel activities rise and fall in accordance with the cell-cleavage cycles (Medina and Bregestovski, 1988). Several cell cycle mutants of *S.pombe* are noted and named for their abnormal sizes and shapes (Russell and Nurse, 1987). It would be interesting to examine the MS channels in these mutant cells.

Now that it is possible to form gigaohm seals and routinely record from the membrane of *S.pombe*, it seems worthwhile to study further the bioelectrics of this species. We are analysing the remaining channels that are gated by voltage but not by membrane tension, since these channels should also be important in the ionic and electric aspects of its

physiology. We have observed unitary currents much smaller than the ones reported, when suctions were applied to on-cell patches. These currents, which seemed to disappear in excised patches or in whole blebs (X.-L.Zhou, unpublished results), should be studied further. Since *S.pombe* may accommodate foreign membrane proteins (Hildebrandt *et al.*, 1989), channels of mammalian or insect origin may also be expressed in *S.pombe* to see how ionic or voltage perturbation through these foreign channels may affect different physiologies, including the control of cell cycle, of *S.pombe*.

Materials and methods

Preparation of blebs

The *S.pombe* strain, *Sp2 H h*^{90} *his3 leu1*-32, was obtained from Dr A.Klar. The methods of cell culture and bleb generation were those of Vondrejz *et al.* (1990), with minor modifications. Briefly, cells were cultured overnight at 30°C in 5 ml of YEP medium (2% glucose, 2% peptone, 1% yeast extract). A second culture was made the following morning in 2 ml YEP medium inoculated with 0.3 ml of the overnight culture at 30°C for 4 h to a final OD_{600} of ~0.3. Cells were then washed twice with and resuspended to OD_{600} of ~0.8 in double-distilled water. 375 μl of this suspension was then mixed with 275 μl of 0.8 M sorbitol and 100 μl of zymolyase stock (2 mg/ml in 0.8 M sorbitol of zymolyase, 60 U/mg, Seikagaku, Kogyo Co., lot no. 8008) and then incubated at 30°C for 30 min with occasional gentle shaking. The cells in this zymolyase-treated culture were then added to 3 ml of solution A (in mM, 120 KCl, 50 $MgCl_2$, 0.1 EGTA, 5 HEPES, adjusted to pH 7.2 with KOH), collected by centrifugation and resuspended in 0.5 ml of fresh solution A. After these treatments, protuberances could be found at one pole of a small number of cells, presumably through cracks in weakened cell wall at the pole. These blebs were dark under light optics and were used for patch-clamp experiments while still physically adhered to the cells, in most instances (Figure 1). In the rare cases when the blebs were detached from the cell body, the characteristics of the MS channels were similar to those of blebs still adhered to cell bodies. Unlike the spheroplast surface of *S.cerevisae* (Gustin *et al.*, 1986), which seems to be exposed as zymolyase gradually erodes the cell wall, the protoplast bleb of *S.pombe* appears to ooze out through the cell poles, where the wall was apparently weakened by the zymolyase. We found that weaker suction and less time are needed to form seal with the surface of *S.pombe* than with that of *S.cerevisae*. A protoplast that actively leaves a shell of cell wall behind appears to have a cleaner surface and may therefore more easliy make a gigaohm seal than a protoplast that passively sheds its wall material during enzyme digestion.

Patch-clamp recording methods

The patch-clamp methods were those of Hamill *et al.* (1981) and Gustin *et al.* (1986), with modifications. 10 μl of the zymolyase-treated cells were added to 0.5 ml solution (in mM, 100 KCl, 20 NaCl, 50 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, adjusted to pH 7.2 with KOH). The patch pipettes (Boralex, Rochester Scientific Co.) were filled with K^+ or Cs^+ solutions (see legend to Figure 4) had a resistance of ~5 MΩ and were coated with nail enamel (Opulence, Cover Girl). All experiments were done at room temperature (19–23°C) with a List-Medical EPC-7 patch-clamp system. Pressure was applied with a syringe and monitored with a pressure transducer (differential type, ± 5 psi, Omega Engineering). Recorded signal was analysed with an INDEC System 11/23 computer after filtering at 1 kHz (8-pole Bessel filter).

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References

Blomberg,A. and Adler,L. (1989) *J. Bacteriol.*, **171**, 1087–1092.
Blomberg,A., Larson,C. and Gustafsson,L. (1988) *J. Bacteriol.*, **170**, 4562–4568.
Brehm,P., Kidokoro,Y. and Moody-Corbett,F. (1984) *J. Physiol.*, **350**, 631–648.
Brown,A.D., Mackenzie,K.F. and Singh,K.K. (1986) *FEMS Microbiol Rev.*, **39**, 31–36.
Delcour,A.H., Martinac,B., Adler,J. and Kung,C.(1989) *J. Membr. Biol.*, **112**, 267–275.
Ghislain,M., Schlesser,A. and Goffeau,A. (1988) *J. Biol. Chem.*, **262**, 17549–17555.
Guharay,F. and Sachs,F. (1984) *J. Physiol.*, **352**, 685–701.
Goffeau,A., Coddington,A. and Schlesser,A. (1989) In Nasim,A, Young,P., and Johnson,B.E. (eds.), *Molecular Biology of the Fission Yeast*. Academic Press, New York, pp. 397–429.
Gustin,M.C. (1991) *Science*, **253**, 800.
Gustin,M.C., Martinac,B., Saimi,Y., Culbertson,M.R. and Kung,C. (1986) *Science*, **233**, 1195–1197.
Gustin,M.C., Zhou,X.-L., Martinac,B. and Kung,C. (1988) *Science*, **242**, 762–765.
Hagan,I.M. and Hyams,J.M. (1988) *J. Cell Sci.*, **89**, 343–357.
Hamill,O.P., Marty,A., Neher,E., Sakmann,B. and Sigworth,F.J. (1981) *Pluger's Arch. Physiol.*, **391**, 85–100.
Hedrich,R., and Schroeder,J.L. (1989) *Annu. Rev. Plant Physiol.*, **40**, 539–569.
Hildebrandt,V., Ramezani-Rad,M., Swida,U., Wrede,P., Grzesiek,S., Primke,M. and Buldt,G. (1989) *FEBS Lett.*, **243**, 137–140.
Hille,B. (1984) *Ionic-channels of Excitable Membranes*. Sinauer Associates Inc., Sunderland, MA, USA.
Hoch,H., Staples,R.C., Whitehead,B., Comeau,J., amd Wolf,E.D. (1987) *Science*, **235**, 1659–1662.
Kobori,H., Yamada,N., Taki,A. and Osumi,M. (1989) *J. Cell Sci.*, **94**, 635–646.
Kubalski,A., Martinac,B. and Saimi,Y. (1989) *J. Membr. Biol.*, **112**, 91–96.
Kung,C. and Saimi,Y. (1985) *Curr. Top. Membr. Trans.*, **23**, 45–66.
Kung,C., Saimi,Y., and Martinac,B. (1990) *Curr. Top. Membr. Trans.*, **36**, 145–153.
Marks,J. and Hyams,J.S. (1985) *Eur. J. Cell Biol.*, **39**, 27–32.
Martinac,B., Beuchner,M., Delcour,A.H., Adler,J. and Kung,C. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 2297–2301.
Martinac,B., Adler,J. and Kung,C. (1990) *Nature*, **348**, 261–263.
Martinac,B., Delcour,A.H., Buechner,M., Adler,J. and Kung,C. (1992) In Ito,F. (ed.), *Comparative Aspects of Mechanoreceptors Systems*. Springer-Verlag, pp. 1–18.
Medina,I.R., and Bregestovski,P.D. (1988) *Proc. R. Soc. Lond. B.*, **235**, 95–102.
Moreno,S., Klar,A., and Nurse,P. (1990) *Methods Enzymol.*, **194**, 795–826.
Morris,C.E. and Horn,R. (1991) *Science*, **251**, 1246–1249.
Nasim,A, Young,P. and Johnson,B.F. (1989) *Molecular Biology of the Fission Yeast*. Academic Press, New York.
Nurse,P. (1991a) *Philos. Trans. R. Soc. Lond. Ser. B.*, **332**, 271–276.
Nurse,P. (1991b) *Nature*, **351**, 245–248.
Russell,P. and Nurse,P. (1987) *Cell*, **49**, 559–567.
Sachs,F. (1988) *CRC Crit. Rev. Biomed. Engng*, **16**, 141–169.
Saimi,Y. and Ling,K.-Y. (1990) *Science*, **249**, 1441–1444.
Saimi,Y., Martinac,B., Gustin,M.C. and Kung,C. (1988) *Trends Biochem. Sci.*, **13**, 304–309.
Saimi,Y., Martinac,B., Delcour,A., Minorsky,P.V., Gustin,M.C., Culbertson,M.R., Adler,J. and Kung,C. (1992) *Methods Enzymol.*, in press.
Vondrejz,V., Pavlicek,I., Kothera,M. and Palkova,Z. (1990) *Biochem. Biophys. Res. Commun.*, **166**, 113–143.
Yang,X.-C. and Sachs,F. (1989) *Science*, **243**, 1068–1071.
Zhou,X.-L., Stumpf,M.A., Hoch,H.C. and Kung,C. (1991) *Science*, 253, 1415–1417.

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