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Mechanosensitive Channels in the Cell Body of *Chlamydomonas*

K. Yoshimura
Department of Biological Sciences, Graduate School of Science, University of Tokyo, Hongo, Tokyo 113-0033, Japan

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Abstract. Mechanosensitive channels appear ubiquitous but they have not been well characterized in cells directly responding to mechanical stimuli. Here, we identified tension-sensitive channel currents on the cell body of *Chlamydomonas*, a protist that shows a marked behavioral response to mechanical stimulation. When a negative pressure was applied to the cell body with a patch clamp electrode, single-ion-channel currents of 2.4 pA in amplitude were observed. The currents were inhibited by 10 μM gadolinium, a general blocker of mechanosensitive channels. The currents were most likely due to Ca^{2+} influxes because the current was absent in Ca^{2+}-free solutions and the reversal potential was 98 mV positive to the resting potential. The distribution of channel-open times conformed to a single exponential component and that of closed times to two exponential components. This mechanosensitive channel was similar to the one found in the flagella in the following respects: both channels were inhibited by Gd^{3+} at 10 μM but not at 1 μM; both passed Ca^{2+} and Ba^{2+}; their kinetic parameters for channel opening were similar. These observations raise the possibility that identical mechanosensitive channels may function both in the behavioral control through the mechanoreception by the flagella and in the regulation of cellular physiology in response to mechanical perturbation on the cell body.

Key words: Mechanosensitive channel — *Chlamydomonas* — Calcium — Flagella — Patch clamp

Introduction

Living organisms perceive mechanical stimuli, such as stretch of the muscle or touch on the body surface, and thereby control their behavior. The primary step in the mechanotransduction is the generation of a receptor potential by mechanoreceptor cells, which increase the ion permeability of membrane and generate receptor currents upon mechanical deformation (Naitoh & Eckert, 1969; Brown, Ottoson & Rydqvist, 1978; Hunt et al., 1978; Rydqvist & Purali, 1993). The receptor current has been thought to pass through the mechanosensitive channels, which open upon receiving mechanical stimuli.

The activities of individual mechanosensitive channels or stretch activated channels have been measured in cells from various sources (*see* Morris, 1990; French, 1992; Sackin, 1995). The first recording was made in cultured skeletal muscle cells by applying negative pressure on the membrane through a patch-clamp pipette (Guharay & Sachs, 1984). With this technique, mechanosensitive channels have been identified also in lens epithelial cells (Cooper, Tang, Rae & Eisenberg, 1986), vascular endothelial cells (Lansman, Hallam & Rink, 1987), *E. coli* (Martinac et al., 1987), budding yeast (Gustin et al., 1988), choroid plexus (Christensen, 1987), kidney cells (Ubl, Murer & Kolb, 1988), *Xenopus* oocytes (Yang & Sachs, 1989), and neuroblastoma cells (Falke & Misler, 1989). However, only few recordings have been reported with those cells that overtly respond to mechanical stimulation (Erxleben, 1989). Possibly, this is because the mechanoreceptor cells usually are very small. Most mechanosensitive channels recorded so far are thought to be involved in volume regulation and morphogenesis rather than motile responses to mechanoreception. Therefore, it has not yet been established experimentally whether true mechanoreceptor cells have mechanosensitive channels similar to those that have been recorded and shown to produce single-channel currents.

Unicellular organisms have been widely used for the study of mechanoreception because their mechanoreception is directly reflected in behavior. For example, *Paramecium* temporarily swims backward when it hits an obstacle with the anterior end. This avoiding reaction is mediated by a depolarizing membrane potential gener-

Correspondence to: K. Yoshimura

ated at the anterior end of the cell (Naitoh & Eckert, 1969; Eckert, 1972). The biflagellated protists *Chlamydomonas* and a related species, *Spermatozopsis*, change swimming directions periodically on mechanical stimulation (Kreimer & Witman, 1994; Yoshimura, 1996). This turning response is apparently due to repetitive impulses produced in the flagella (Yoshimura, 1996).

In this study, we report that a negative pressure applied on the membrane of the cell body in *Chlamydomonas* elicited single channel currents. These channels were compared to those that produce repetitive impulses in the flagella upon mechanical stimulation and found to be similar or identical in terms of ion selectivity, sensitivity to Gd^{3+}, and kinetics of channel openings.

Materials and Methods

Cell Culture and Solutions

A cell-wall-deficient mutant of *Chlamydomonas reinhardtii*, *cw2*, was used. Cells were cultured in the liquid TAP medium (Gorman & Levine, 1965) under 12 hr:12 hr light:dark conditions. The cells were washed three times with a solution containing 1 mM KCl, 0.3 mM $CaCl_2$, 0.2 mM EGTA, and 5 mM HEPES (Harz & Hegemann, 1991), pH adjusted to 7.1 with N-methylglucamine. This solution was used as the bath and the pipette solution. EGTA was omitted and $CaCl_2$ was reduced to 0.1 mM when $GdCl_3$ was added.

Stimulation and Observation

Negative pressure (50–130 cm H_2O) was applied to the cell membrane through a suction pipette and measured with a pressure gauge (PD200GA, Kyowa Dengyo, Tokyo, Japan). Light microscopic observations were carried out with an inverted microscope (MD, Nikon, Tokyo, Japan). The cells were illuminated with > 600 nm light to avoid photoresponse. All experiments were carried out at room temperature (21–25°C).

Electrophysiological Methods

The apparatus and the methods for recording membrane currents were essentially as reported previously (Yoshimura, 1994). Electrodes were prepared by pulling borosilicate glass capillary (outer diameter: 1.5 mm; inner diameter: 0.86 mm; GC150, Clark Electromedical Instruments, Reading, UK) with a needle puller (PP-83, Narishige, Tokyo, Japan). Their tips were heat polished (MF-83, Narishige, Tokyo, Japan). Use of micropipettes with a thick heat-polished glass wall was important for preventing cells from being ruptured when negative pressure was applied. The electrodes filled with the bath solution had resistances of 170–280 MΩ (tip opening: 0.7–1.0 μm; bubble number: 5.2–5.9). Their resistances were 5–7 MΩ if filled with 150 mM KCl. The resistance increased 2–3-fold when the electrode was pressed onto the cell surface and a sustained negative pressure was applied. Gigaohm seal was not attained. This is probably due to extracellular materials remaining on the cell membrane. The captured cell was lifted close to the surface of the bath solution to reduce the electrical noise and to increase the time resolution of the recording.

Current was measured with a patch clamp amplifier (EPC 7, List-Medical, Darmstadt, Germany; Axopatch 200B, Axon Instruments, CA) and filtered with a 3 kHz low-pass Bessel filter. The current and pressure data were stored on magnetic tapes with a DAT recorder (59ES, Sony, Tokyo, Japan) which had been modified so as to record direct currents. The stored records were digitized at 10 kHz with an A/D converter and a microcomputer. Power density spectra were obtained with an FFT analyzer (AD3524, A and D Co., Tokyo, Japan). The spectrum of the background noise was subtracted from that of the experimental data.

The digitized data were filtered with a Gaussian filter (1 kHz) and analyzed with AxoGraph 3.03 (Axon Instrument, Foster City, CA). The currents flowing inward (i.e., from the pipette into the cell) are presented as negative value or downward current. For the analysis of the kinetics of current openings, transitions between the open and closed states were detected as crossings of a threshold level set halfway between the open and closed channel levels (Colquhoun & Sigworth, 1983). The number of channels was estimated by the methods of Colquhoun and Hawkes (1983). Recorded data were not used for kinetic analysis if the probability that more than two channels were present was >1%.

Results

Patch-clamp Examination of the *Chlamydomonas* Cell Body

Chlamydomonas repetitively changes its swimming direction when it is stimulated mechanically (Yoshimura, 1996). This response is thought to be brought about by impulses that occur periodically in the flagella. In the previous study, we measured the currents by applying negative pressure (5–20 cm H_2O) to the paired flagella through an electrode (Yoshimura, 1996; Fig. 1*A*, *see* Fig. 5*C*). The mechanism for generation of repetitive currents is probably localized in the flagella because such currents were not generated when the cell was caught on an electrode with its flagella outside of the electrode (Fig. 1*B*). We noticed, however, that flickering currents were sometimes observed in this configuration, especially when a higher pressure was applied (about 100 cm H_2O). We thus modified the shape of the electrode tip to enable single channel recording.

The report here summarizes recordings from >300 cells. There are no previous reports of single-channel activities recorded directly from the *Chlamydomonas* body membrane. Extensive efforts have been made to attain GΩ seal but to no avail. This includes the examination of mutant strains, digestion of the cell surface, and modification of the culture conditions. I was nonetheless encouraged by the repeated appearance of unitary currents upon suction in more than 80% of the cases when >500 MΩ seals were obtained. Ion selectivity, non-zero reversal potential and other observations (below) showed that these are biologic membrane currents and not artifacts of the loose seal. To my knowledge, this is the first report of unitary currents through mechanosensitive

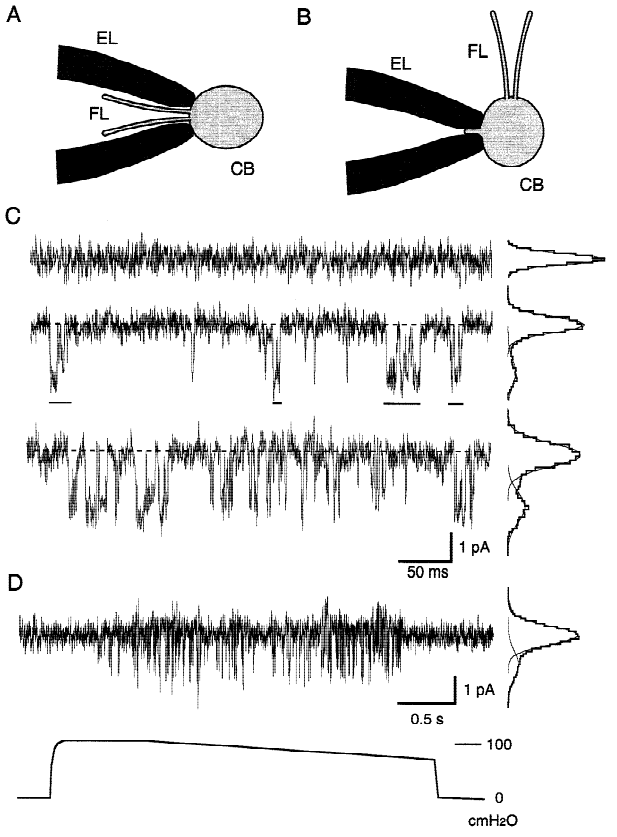

Fig. 1. (*A*) Schematic diagram of the configuration of a *Chlamydomonas* cell captured on a suction pipette with its flagella (FL) inside of the electrode (EL). Under this configuration, repetitive impulses were generated in the flagella. (*B*) When a cell was captured with its flagella outside of the pipette, single-channel currents of mechanosensitive channel were observed on the cell body (CB) (*C*) Single-channel currents upon applying negative pressure on the cell body of *Chlamydomonas*. The suction pipette was held at the same potential as that of the bath solution. Currents in the absence (top) and presence of pressure (middle: 85 cm H_2O; bottom: 90 cm H_2O) are shown. The channel openings often occurred in bursts (bars). The broken line indicates the closed-state current levels. The negative (downward) current represents the current flowing into the cell. (*D*) The response to a pressure change. The pressure was increased steeply from 0 to 105 cm H_2O, decreased linearly to 75 cm H_2O and dropped to 0 cm H_2O. In *C* and *D*, the amplitude histograms are shown at the right side. The histogram can be fitted to two components of normal distributions, which represent the closed and open states of the channels.

channels in any protists, though protists' behavioral responses to mechanical stimuli have long been observed (Jennings, 1906).

The techniques that I was able to develop on this previously unexplored cell surface have limitations. The recordings were usually limited to <60 sec. This may represent the inactivation of the channel or relaxation of the membrane tension. It was not possible to apply strong suctions since cells were drawn into the pipette. All experiments were repeated at least 10 times unless otherwise mentioned.

Currents Induced by Mechanical Stimulation of Cell Body

When a negative pressure was applied on the cell body through patch-clamp pipette, square-wave currents of 2.4 ± 1.5 pA (20 cells, mean ± SD, for all statistics below) were resolved (Fig. 1*C*). The direction of the current was inward (i.e., into the cell). This current is driven by the cell's resting potential because the potential of the pipette was held the same as that of the bath. Since the currents occurred only when a negative pressure was applied (Fig. 1*C* and *D*), it is likely that they represent channels activated by mechanical stimuli. The absence of the current prior to and just after the beginning of the application of pressure was not due to poorer seal because the seal resistance monitored by application of electric pulses was constant (*data not shown*).

Suction-induced channel activity was reversible when a reasonably high seal resistance was attained (Fig. 1*D*). When the seal resistance was low, the channel activity disappeared soon after the onset of pressure because the cell visibly slipped into the pipette. This suggests that the channel activation was due to the membrane extension rather than some movement between the cell membrane and the glass surface which might occur under the loose seal condition.

In mechanosensitive channels of other organisms previously studied, the open probability depends on the magnitude of the stimuli (Guharay & Sachs, 1984; Sackin, 1989). When a ramp of pressure was applied on a *Chlamydomonas* cell, the open probability increased from nil to about 0.2 at pressures just beyond the threshold (Fig. 1*C* and 2*A*). At higher pressures when the cell membrane slid into the pipette further increases in the open probability were not observed.

If the currents were due to mechanosensitive channels, one could expect gadolinium, which blocks cation-selective mechanosensitive channels at micromolar concentrations (Yang & Sachs, 1989; Franco & Lansman, 1990), would inhibit them. Thus, the effect of Gd^{3+} on the pressure-induced currents was tested on 17 independent patches in the presence of 1 or 10 μM Gd^{3+} in the bath and pipette solution. None of the 7 cells examined produced pressure-induced currents in the presence of 10 μM Gd^{3+} (Fig. 2*B*). On the other hand, all of the 10 cells measured in the presence of 1 μM Gd^{3+} produced mechanosensitive single channel currents (Fig. 2*B*). Therefore, the pressure-induced currents were inhibited by Gd^{3+} at a concentration similar to that effective for other known mechanosensitive channels.

The channel often opened in bursts although individual opening events were also present (Fig. 1*C*). The open-time distribution can be fitted to a single exponential component with a time constant of $\tau_{open} = 1.4 \pm 0.6$ msec ($n = 12$, Fig. 3*A*). The closed-time distribution can be fitted to the sum of two exponential components

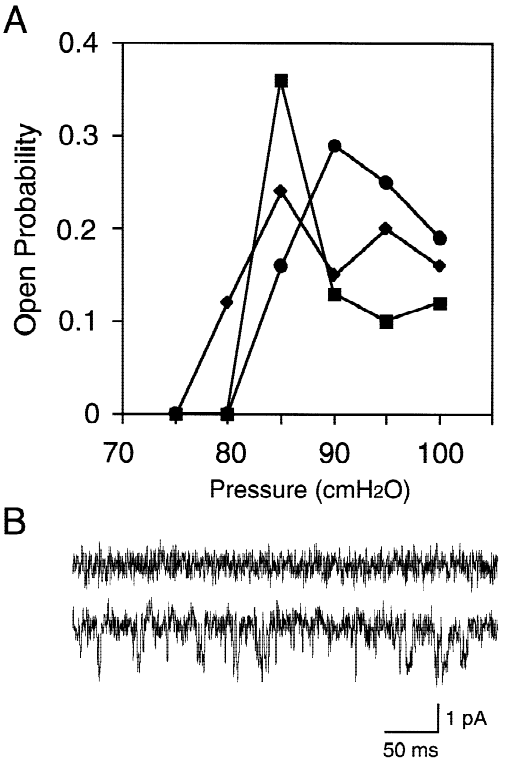

Fig. 2. (*A*) The change in open probability when a ramp of pressure such as in Fig. 1*D* was applied. The channels opened only when the pressure exceeded a threshold level. The three symbols refer to three independent experiments. (*B*) Effect of Gd^{3+}. Channel activities at constant 95 cm H_2O suction were completely inhibited in the presence of 10 μM Gd^{3+} (top) but not in the presence of 1 μM (bottom).

(Fig. 3*B;* Sigworth & Sine, 1987). The shorter closure (τ_{short} = 1.3 ± 0.5 msec; Fig. 3*B*) probably corresponds to the closure within bursts and the longer one (τ_{long} = 41 ± 38 msec) corresponds to the closure among bursts and individual opening events. When the closures shorter than 3 msec were neglected, the time constant was τ_b = 3.8 ± 3.4 msec (Fig. 3*C*). This constant can be regarded as the mean burst duration. The dependence of the channel kinetics on the magnitude of stimulus was not explored because we could not change the channel activity over a wide range (*see above*).

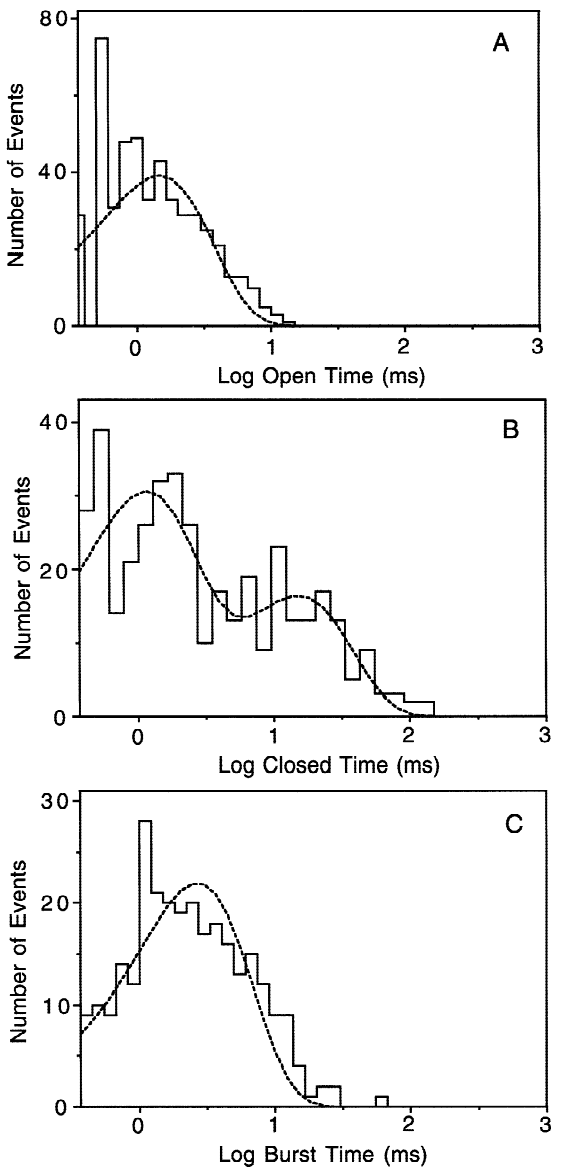

Fig. 3. Open and closed time distributions of the currents of the mechanosensitive channels on the cell body. (*A*) The distribution of channel open times (n = 542) can be fitted to a single exponential function (broken line: decay time constant: 1.5 msec). (*B*) The distribution of channel closed times. It can be fitted to two exponential functions with time constants of 1.1 and 15 msec. (*C*) The histogram of burst duration can also be fitted to a single exponential curve (mean duration: 2.7 msec).

Ions Passing Through the Channels

To characterize the ions passing through the channels, we modified the ionic composition of the solution and applied voltage steps through the electrode. The probable candidates of the ions flowing inwardly are Ca^{2+} and K^+. When the K^+ was omitted from the medium or replaced with N-methylglucamine, the channel current did not change significantly (2.4 ± 0.2 pA, n = 7; Fig. 4*A*). In contrast, when Ca^{2+} was depleted, the activity of the mechanosensitive channel disappeared completely (Fig. 4*B*). When Ca^{2+} was substituted with Ba^{2+}, normal channel activities were observed (Fig. 4*C*). There was no significant difference in the channel kinetics observed in the presence of Ba^{2+} and Ca^{2+}: the amplitude was 2.1 ± 0.6 pA and the mean burst duration (τ_b) was 3.3 ± 1.5 msec (n = 4). These observations suggest that the channel is almost equally permeable to Ca^{2+} and Ba^{2+}.

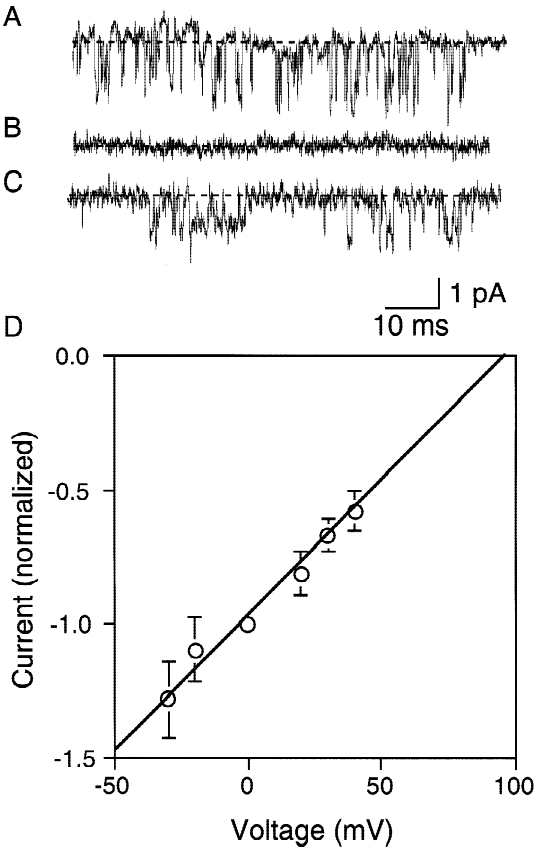

Fig. 4. Change in the activity of mechanosensitive channels of the cell body under various ion compositions and holding potential. The bath and the electrode contained the same solution. Channel activities observed when K^+ was omitted (*A*), when Ca^{2+} was omitted (*B*) and when Ca^{2+} was replaced by Ba^{2+} (*C*). The broken lines indicate the current levels of the closed-state. (*D*) The amplitude of the unitary current as a function of applied potential. The amplitude is normalized by that at 0 mV because the original value varied with the seal resistance. The potential is relative to the resting potential. The mean and the standard deviation from 5 experiments are shown. The slope of the regression line was 1.0×10^{-2} mV^{-1}.

The relationship between the holding potential and the amplitude of the unitary current was almost linear as shown in Fig. 4*D*. It was not possible to resolve single-channel currents at more positive potential because of the substantial leak current. The current was normalized because the amplitude at the resting potential varied among preparations. The reversal potential calculated from the regression line was +98 mV relative to the resting potential. The slope conductance was 24 pS if we assume that the amplitude at the resting potential was 2.4 pA.

Comparison with the Currents Produced by Mechanical Stimulation on Flagella

Chlamydomonas cells have been found to produce repetitive impulses on mechanical stimulation of the flagella (Figs. 1*A* and 5*C*). Most of the impulse current is passed by mechanosensitive channels rather than voltage-gated channels (Yoshimura, 1996). We examined whether similar channels are responsible for the mechanoreception by the flagella and the cell body by comparing the ion permeability, the sensitivity to Gd^{3+}, and the kinetics of the channel openings.

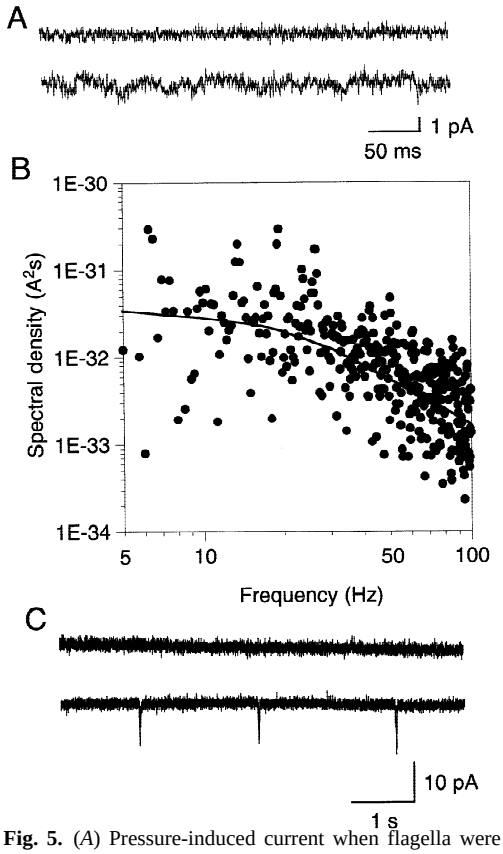

Fig. 5. (*A*) Pressure-induced current when flagella were sucked into the pipette in the presence of Ba^{2+} (no Ca^{2+}). The currents in the absence (top) and presence of pressure (bottom). Fluctuation of current appeared in the presence of mechanical stimulation. (*B*) Power density spectrum of the current in the presence of pressure. The distribution can be fit to single Lorentzian curve (continuous line) with a corner frequency (f_c) of 32.6 Hz. This frequency indicates the presence of channel activity with a time constant of 4.9 msec ($=1/2\pi f_c$). (*C*) Effect of Gd^{3+} on the pressure-induced impulses produced in the flagella. The impulses were blocked at 10 μM (top) but not at 1 μM (bottom).

The effects of extracellular ions on the flagellar impulses were almost identical with those observed with the single-channel currents on the cell body. Namely, the flagellar currents like the cell body currents have been found to depend on the presence of external Ca^{2+}; Ba^{2+} can substitute for Ca^{2+}, although it abolishes repetitive firing of spikes (Fig. 5*A; see also* Yoshimura, 1996). Furthermore, both currents were found to be inhibited by 10 μM Gd^{3+} but not by 1 μM Gd^{3+} (Fig. 5*C*).

The kinetic parameters of the mechanoreceptor channels in the flagella were determined by fluctuation analysis (Katz & Miledi, 1970; Anderson & Stevens,

1973) on the data recorded in the presence of Ba^{2+}, when cells produce continuously fluctuating currents (Fig. 5*A*). In this analysis, we assumed that the observed fluctuation was solely due to the channel activity. The power spectra of this fluctuation were fitted to a single Lorentzian curve ($f = S(0)/(1 + (f/f_c)^2)$; Fig. 5*B*) with the corner frequency of $f_c = 35.1 \pm 3.5$ Hz (5 cells). This indicates the presence of a channel activity with a time constant of $\tau_f = 4.6 \pm 0.5$ msec ($= 1/2\pi f_c$). Time constants obtained from fluctuation analyses have been shown to agree well with the mean burst durations measured in single-channel recordings (Trautmann & Siegelbaum, 1983). In the present case, the mean burst duration of the single-channel currents was $\tau_b = 3.8 \pm 3.4$ msec. This value is in good agreement with τ_f.

Discussion

We have shown that *Chlamydomonas* produces currents when its cell body was subjected to suction via a micropipette. The appearance of unitary current, the dependence of the activity upon extracellular ion composition, and the non-zero reversal potential indicate that the currents are produced by ion-selective channels. The channels appear to be activated by the stretch of the membrane because the currents were observed only in the presence of negative pressure and because the response was reversible only when the seal resistance was relatively high but not when the cell slid into the pipette. The currents were inhibited by Gd^{3+}, a blocker of mechanosensitive cation channels, at a micromolar range. These observations indicate that the ion currents observed here are due to the activity of mechanosensitive channels, and not an artifact deriving from a loose seal. This is the first report on the activity of the single-mechanosensitive channels in protists to the best of our knowledge.

The mechanosensitive channels of *Chlamydomonas* cell body appears to be permeable to divalent cations because the activity was not observed in the absence of extracellular Ca^{2+} and because Ca^{2+} can be replaced by Ba^{2+}. The reversal potential of 98 mV positive to the resting potential supports the idea that Ca^{2+} is the major current carrier rather than K^+. The finding that channel currents remained normal when extracellular K^+ was depleted also supports this idea. Thus, the mechanosensitive channels in *Chlamydomonas* is similar to those in other protists, such as *Paramecium, Stylonychia,* and *Stentor* (Naitoh & Eckert, 1969; Wood, 1970; de Payer & Machemer, 1978), all of which produce Ca^{2+}-dependent receptor potential on mechanical stimulation.

The mechanosensitive channels of *Chlamydomonas* share several characteristics with those in higher animals that have been studied in detail. First, the channel activity is inhibited by Gd^{3+} at micromolar concentrations (Yang & Sachs, 1989). Second, channels tend to open in bursts (Guharay & Sachs, 1984). Third, the channel open time is in the order of millisecond in both *Chlamydomonas* and animal cells. The kinetic data presented here are possibly nonstationary values because the channel activity tended to decline during recording. Finally, the conductance of the *Chlamydomonas* channel may be 30–50 pS, if we assume that 50–70% of the membrane current is recorded under the loose-seal condition. This is within the range of the conductance of the mechanosensitive channels reported in animal cells (*see* French, 1992). The conductance was measured at 0.1 mM free Ca^{2+}, which is significantly lower than the concentration of K^+ usually used for studies of potassium-permeable channels. A significantly high conductance at low ion concentration may be essential for induction of physiologically important Ca^{2+} influx in a *Chlamydomonas* cell which lives in fresh water. *Chlamydomonas* channels probably differ in property from the mechanosensitive channels in *E. coli,* which have a conductance as large as 1–3 nS and time constants of 6 and 37 msec (Martinac et al., 1987; Sukharev et al., 1993; Blount et al., 1996).

The mechanosensitive channels present in the cell body were found to be similar to those found in the flagellar membrane, which are important for the regulation of cell behavior. Features shared by the two kinds of channels include: inhibition by Gd^{3+} at 10 μM but not at 1 μM; permeability to both Ca^{2+} and Ba^{2+}; kinetics of channel opening (τ_b and τ_f). Although the resolution of the recording is limited in the present study, especially in the kinetic analysis, these similarities suggest that the mechanosensitive channels in flagellar and cell-body membranes are identical. This study, therefore, provides an experimental support for the linkage between the pressure-activated single-channel currents and the behavioral response in mechanosensing cells.

The physiological function of the mechanosensitive channels present on the cell body is not fully understood. One possibility is that they function in detection of the swelling of the cell; *Chlamydomonas* cells, living in fresh water, are always exposed to hypo-osmotic stress. The cell survives even when the cell wall is shed off during mating process. The detection of swelling by the mechanosensitive channels may activate some machinery for the protection against swelling. Because *Chlamydomonas* is particularly suited for genetic and molecular biological analysis, we expect that the physiological meaning of the mechanosensitive body channel as well as its relationship to the flagellar channel will be made clear by generating mutants defective in the mechanoreception.

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