The Journal of Experimental Biology 212, 2767-2772
Published by The Company of Biologists 2009
doi:10.1242/jeb.031278

Thermo-sensitive response based on the membrane fluidity adaptation in *Paramecium multimicronucleatum*

Taichi Toyoda[1], Yoshinori Hiramatsu[2], Toshiaki Sasaki[1] and Yasuo Nakaoka[1,2,*]

[1]Biophysical Dynamics Laboratories, Graduate School of Frontier Bioscience and [2]Division of Biophysical Engineering, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan

*Author for correspondence (nakaoka@bpe.es.osaka-u.ac.jp)

Accepted 9 June 2009

SUMMARY

Relationships between the thermo-sensitive response and membrane lipid fluidity were studied using a ciliated protozoan, *Paramecium multimicronucleatum*. *Paramecium* elicits a transient membrane depolarization in response to a cooling stimulus (temperature drop). The depolarization amplitude was largest when the cooling stimulus was started from the culture temperature, whilst when cooling started at a temperature more than 5°C higher or lower than the culture temperature, only a small depolarization was induced. Therefore, the cooling-induced response was dependent on the culture temperature and its sensitivity to the cooling stimulus was highest at the culture temperature. Membrane fluidity measurements of living cells using the fluorescent dye 6-lauroyl-2-dimethylaminonaphthalene (laurdan) showed that the fluidity measured at the culture temperature was almost constant irrespective of the temperature at which the cells had been cultured and adapted, which is consistent with homeoviscous adaptation. The constant fluidity at the culture temperature quickly decreased within a few seconds of application of the cooling stimulus, and the decreased fluidity gradually readapted to a constant level at the decreased temperature within 1 h. When the constant fluidity at culture temperature was modified by the addition of procaine or benzyl alcohol, the cooling-induced depolarization was completely abolished. These results suggest the possibility that the adaptation of fluidity to a constant level and its quick decrease below the constant level activate cooling-sensitive channels to elicit the transient depolarization.

Key words: temperature, adaptation, cooling-induced response, membrane fluidity, laurdan fluorescence, *Paramecium*.

INTRODUCTION

When *Paramecium* cells are placed in a temperature-gradient vessel, they accumulate at the temperature at which they have been cultured as though they remember the culture temperature (Jennings, 1906; Mendelssohn, 1895). Such an accumulation is caused by thermo-sensitive behaviour within the temperature gradient (Nakaoka and Oosawa, 1977; Oosawa and Nakaoka, 1977). Cells swimming away from the culture temperature increase the frequency of directional changes, while cells swimming to the culture temperature decrease the frequency of directional changes. Therefore, *Paramecium* cells seem to detect the temperature gradient as a temporal change of temperature by swimming in the temperature gradient. In fact, when the cells are subjected to a temporal change of temperature starting from the culture temperature, they show a transient increase in the frequency of directional changes whilst swimming (Nakaoka and Oosawa, 1977). This increase in the frequency of directional changes is induced by a transient depolarization of the membrane potential, which accompanies the activation of Ca^{2+}-permeable channels, although the cooling-sensitive Ca^{2+} channels (Kuriu et al., 1996) are different from the heating-sensitive Ca^{2+} channels (Imada and Oosawa, 1999; Nakaoka et al., 1987; Tominaga and Naitoh, 1994) in terms of ionic selectivity. Hence, activation of these thermo-sensitive channels is related in some way to the culture temperature to which cells have been adapted.

Environmental temperature directly affects the physical properties of membranes, particularly in unicellular organisms. A temperature drop or rise induces, respectively, a decrease or an increase in membrane lipid fluidity, which alters the activity of membrane proteins. In response to such changes, many cells can adapt to a temperature change by altering their lipid composition and restore membrane fluidity to a constant level *via* a process termed homeoviscous adaptation (Anderson et al., 1981; Hazel, 1995; Martin et al., 1976; McKinley and Hazel, 2000; Sinensky, 1974; Soicic et al., 1992; Williams and Somero, 1996). In most cases, however, this membrane fluidity adaptation has been inferred from studies of extracted lipid components. We aimed to examine the homeoviscous adaptation in living cells.

In order to measure the membrane fluidity of living cells, we used the lipophilic fluorescent probe laurdan. Laurdan in the lipid phase shows spectral sensitivity with a red shift of the emission maximum when passing from a gel to a liquid crystalline phase (Chong and Wong, 1993; Parasassi et al., 1990; Parasassi et al., 1991; Sheffield et al., 1995). Based on this property, laurdan has been used to estimate the fluidity of the membrane in various living cells (Chapman et al., 1995; Harris et al., 2001; Mamdouh et al., 1998; Palleschi and Silvestroni, 1996; Sasaki et al., 2006; Vest et al., 2004; Yu et al., 1996).

The temperature dependence of the membrane fluidity measured in this study by laurdan fluorescence imaging suggests the possibility that a rapid change in fluidity activates thermo-sensitive channels to elicit membrane depolarization.

MATERIALS AND METHODS

Chemicals

Laurdan (6-lauroyl-2-dimethylaminonaphthalene) was purchased from Molecular Probes (Eugene, OR, USA). All other reagents were from Wako Chemicals (Osaka, Japan).

Cell culture

Paramecium multimicronucleatum Powers and Mitchell 1910 cells were cultured in a hay infusion inoculated with *Klebsiella pneumoniae*. The culture temperature was kept constant by incubation in a water bath. *Paramecium multimicronucleatum* cells in the stationary phase, 7–14 days after inoculation, were collected by low speed centrifugation and suspended in a standard solution containing (mmol l^{-1} final concentration): 0.25 $CaCl_2$, 2 KCl, 0.5 $MgCl_2$ and 2 Tris-HCl (pH 7.2). For adaptation of the cells, the suspension was left for at least 2 h in a water bath at the culture temperature.

Frequency of directional changes in swimming

Paramecium multimicronucleatum cells suspended in standard solution were placed in a rectangular, thin vessel the temperature of which was controlled by water flow beneath the vessel. Swimming behaviour was recorded with a camera mounted on the vessel (Nakaoka and Oosawa, 1977). Swimming tracks of 2 s duration were taken for 50–100 cells simultaneously, and the fraction of tracks that showed directional changes was counted. The frequency of directional changes is expressed as the fractional number per second.

Intracellular recording of membrane potential

Cells were deciliated by incubation in a standard solution containing 6% ethanol and gently pipetted for 0.5–1 min and transferred to the standard solution without ethanol. The method of recording was similar to that described previously (Nakaoka et al., 1991). The electrode was filled with 0.1 mol l^{-1} KCl and the resistance was 100–150 MΩ. The deciliated cells were placed in a glass vessel mounted on an inverted microscope and electrodes were inserted from above. The temperature was changed by switching the water flow beneath the vessel, and monitored with a thermistor probe placed in the vessel (Nakaoka et al., 1987).

Laurdan labelling and fluorescence imaging

Methods were similar to those detailed in a previous report (Sasaki et al., 2006). In order to stop cellular movements during fluorescence imaging, *P. multimicronucleatum* cells were deciliated as described above. The deciliated cells were labelled in a standard solution for 40 min in the dark with 15 μmol l^{-1} laurdan from a 2 mmol l^{-1} stock solution in dimethylformamide. During these procedures the temperature of the cell suspension was kept at the culture temperature. Laurdan-labelled cells were dropped on a coverslip and the coverslip was adhered to the lower side of a temperature-controlled glass vessel that was placed on the stage of an inverted epifluorescence microscope (Olympus IX-70 with IX-FLA, Tokyo, Japan). The temperature of the vessel was controlled by the water flow from the water bath and was monitored with a thermistor probe placed near the specimen. It was confirmed that the deciliation procedure had no effect on the fluorescence ratio imaging.

For fluorescence ratio imaging, excitation light was supplied from a 75 W xenon lamp. An electric shutter (Copal No. 0, Tokyo, Japan) and neutral density filter which cut out 12% of the excitation intensity were placed in the excitation light path. Laurdan was excited with a dichroic mirror (bandpass 360–375 nm) and emitted fluorescence was divided into two light paths with double-view optics (Hamamatsu Photonics A4313, Hamamatsu, Japan). Two images passed through bandpass filters (Omega Optical, VT, USA) of 440 nm/20 nm and 495 nm/20 nm were simultaneously recorded as a single image with an EB-CCD camera (Hamamatsu Photonics C7190-20) coupled to an image intensifier (Videoscope VS4-1845, VA, USA). The image averaged over 8 frames during 0.27 s was stored and calculated with an image processor system (Argus-20 and Aqua Cosmos, Hamamatsu). In order to subtract cellular autofluorescence, the cell image without laurdan was set at no fluorescence by reducing the gain of the image intensifier. The same instrumental condition was adopted for the fluorescence measurement in the presence of laurdan.

The generalized polarization (GP) for each pixel was calculated using $GP=(I_{440}-I_{495})/(I_{440}+I_{495})$, where I_{440} and I_{495} are the fluorescence intensities measured at the emission maximum of laurdan that are characterized by the gel and the liquid crystalline phases, respectively (Parasassi et al., 1990; Parasassi et al., 1991). GP distribution was obtained from the histograms of GP image, and GP value was determined from the mean of the distributions.

RESULTS

Thermo-sensitive response in relation to culture temperature

Paramecium cells cultured at 18°C, 23°C or 28°C were impaled with glass microelectrodes and membrane potential was recorded at various temperatures for 1–3 min; the cooling stimulus of a temperature drop of 5°C was then applied. When the cooling stimulus was started from the culture temperature, a large depolarization was transiently elicited (Fig. 1A). In contrast, when cooling started from 5°C higher or lower than the culture temperature, only a small depolarization was elicited. Such a cooling stimulus of a constant temperature drop was started from various temperatures and the amplitudes of the elicited depolarizations were measured (Fig. 1B). The maximum depolarization was elicited by a cooling stimulus that started from the culture temperature, while the depolarization amplitude was decreased when the cooling stimulus was started at a higher or lower temperature than the culture temperature. Sensitivity to the cooling stimulus is, therefore, highest at the culture temperature to which cells have been adapted.

Adaptation time course in cooling-sensitive response

When a cooling stimulus is applied at the culture temperature, *Paramecium* cells swimming in a standard solution show a transient increase in the frequency of directional changes (Nakaoka and Oosawa, 1977), which corresponds to the magnitude of the induced transient depolarization. In contrast a cooling stimulus started either 5°C higher or lower than the culture temperature induces only a small increase in the frequency of directional changes. Based on these results indicating a thermo-sensitive response, we tested the time required to adapt to a shift in culture temperature by recording the swimming response. After a shift of the culture temperature by 5°C from a start point of 18°C and 23°C, the cooling stimulus of a constant temperature drop from the shifted temperature was applied at various times and transient increases in the frequency of directional changes were measured. The increase in frequency in each case was small at first, then increased gradually, almost reaching saturation within 1–2 h (Fig. 2). Consistent with these results, we confirmed that the transient depolarization in response to the cooling stimulus starting from the shifted temperature increased gradually and reached saturation within a similar interval (data not shown). These results suggest that the cooling-sensitive response recovers with adaptation to the shifted temperature.

Membrane fluidity and culture temperature

Paramecium cells were labelled with laurdan at the culture temperature and the GP values indicating membrane fluidity were measured at various temperatures. In order to avoid strong

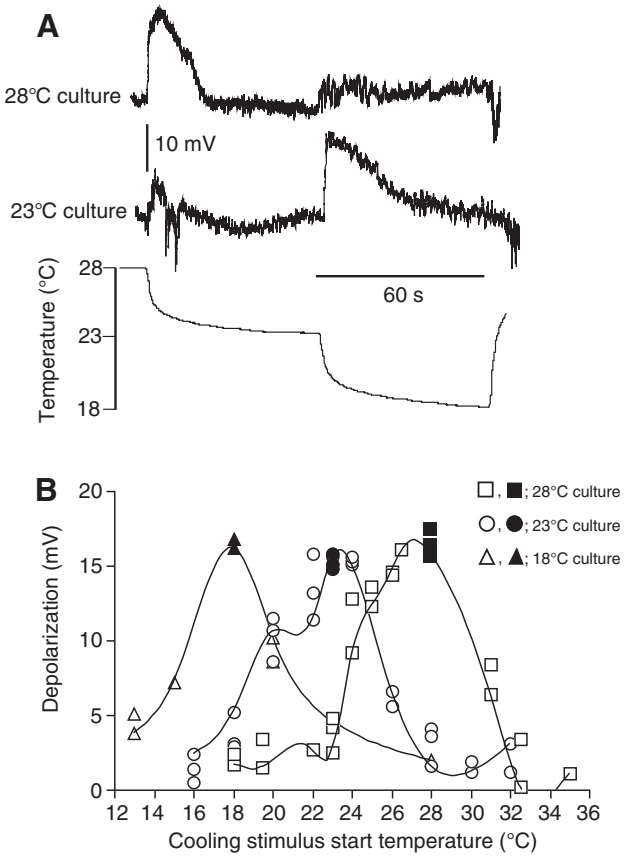

Fig. 1. Cooling-induced depolarization applied at various temperatures. Membrane potentials of cells cultured at 18°C, 23°C or 28°C were initially recorded at various temperatures for 1–3 min, then the cooling stimulus (temperature drop of 5°C) was applied and the transient depolarization recorded. (A) Relationship between the transient depolarization and the culture temperature. The cooling stimulus (bottom trace) was applied to cells cultured at either 28°C (top trace) or 23°C (middle trace). (B) Transient depolarization induced by the cooling stimulus started from various temperatures. Abscissa shows the temperature at which the cooling stimulus was started. Filled symbols indicate that the temperature at which the cooling stimulus started corresponds to the respective culture temperature as indicated in the inset.

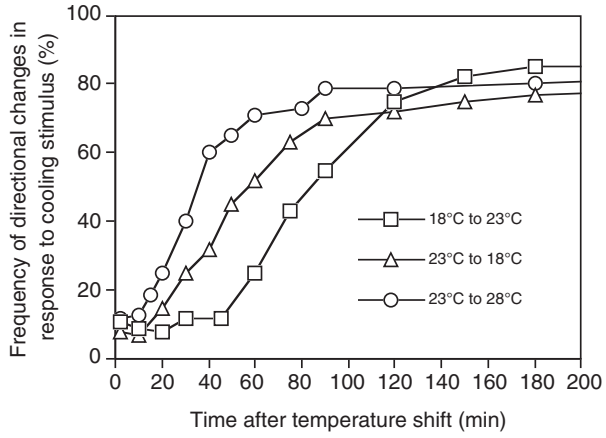

Fig. 2. Adaptation of the cooling-induced swimming response after a shift of the culture temperature. After the culture temperature was shifted, a fraction of the cells were transferred to a thin vessel temperature controlled at the shifted temperature, and left for 1min; a rapid temperature drop of 5°C was then applied at various times after the shift of culture temperature, which is indicated as time zero on the abscissa. The frequency of directional changes on the ordinate indicates the fraction of swimming tracks that show directional changes in response to the cooling stimulus as explained in Materials and methods.

fluorescence from the food vacuole, GP values were calculated within an area of the anterior portion where food vacuoles were scarce (see circled area in Fig. 3A). Calculated GP values were distributed widely as a symmetrical histogram and the histogram shifted quickly to higher levels when the measurement temperature was decreased from the culture temperature (Fig. 3A). Based on these results, the GP value following the change in temperature was determined from the mean of the GP distribution within the circled area of the GP image.

For cells cultured at 18°C, 23°C or 28°C, GP values decreased with an increase in measurement temperature (Fig. 3B). These temperature-dependent curves were the same whether the GP values were measured for an increase or a decrease in temperature. In each curve, the temperature-dependent change in GP value was largest near the culture temperature, especially just below the culture temperature. When the measurement temperature corresponded to the culture temperature, respective GP values were almost constant at 0.18, irrespective of the culture temperature. Therefore, these results indicate that the homeoviscous adaptation is established in the membrane of living *Paramecium* cells.

Adaptation time course for membrane fluidity

In order to investigate the time required for membrane fluidity adaptation, deciliated *Paramecium* cells cultured at 23°C were labelled with laurdan by incubation at 23°C; the incubation temperature was then shifted to 28°C and the GP values were measured successively. The GP value measured at 28°C initially decreased at the elevated temperature but then increased gradually and reached a constant of 0.18 (Fig. 4A). In contrast, when the incubation temperature was shifted from 23°C to 18°C, the GP value measured at 18°C increased initially and then decreased gradually to the adapted GP value of 0.18 (Fig. 4B). For a temperature shift from 23°C to either 28°C or 18°C, the time for adaptation to the shifted temperature was about 1 h. These time courses of GP changes suggest that the membrane fluidity is regulated to a constant level during adaptation to the shifted temperature.

Membrane fluidity and the thermo-sensitive response

In order to investigate the relationship between the thermo-sensitive response and membrane fluidity, chemicals that inhibit the thermo-sensitive response were tested. It was found that addition of procaine, benzyl alcohol or $MgCl_2$ abolished the transient depolarization induced by the cooling stimulus (Fig. 5).

The effect of these chemicals on membrane fluidity was tested by GP measurements at the culture temperature, 23°C (Fig. 6A). Addition of procaine increased the GP value, suggesting a decrease in membrane fluidity. In contrast, addition of either benzyl alcohol or $MgCl_2$ decreased the GP value, suggesting an increase in membrane fluidity. Subsequently, the effects of these chemicals on the cooling-induced increases in GP value were measured after application of the cooling stimulus (temperature drop from 23°C to 18°C). The extent of the increase in GP values in the presence of any of these chemicals was about a half that of the control condition (Fig. 6B).

DISCUSSION

Paramecium cells swimming in a temperature-gradient vessel accumulate at the temperature at which they have been cultured and

adapted (Nakaoka and Oosawa, 1977). Such an accumulation suggests that *Paramecium* cells have some memory about the culture temperature. In this study, the culture temperature was found to be related to the amplitude of the cooling-induced depolarization. The amplitude was greatest when the cooling stimulus was applied at the culture temperature (Fig. 1). That is, the sensitivity to the cooling stimulus was the highest at the culture temperature. Based on such characteristics, we found the time for adaptation from 23°C to 18°C or 28°C to be about 1.5 h (Fig. 2). Over this period, *Paramecium* cells readapt to the shifted temperature and form some memory of the culture temperature within the cell.

Temperature adaptation has been shown to accompany alterations of the membrane lipid composition in *Paramecium* cells (Hennessey and Nelson, 1983). This compositional alteration allows a constant level of membrane fluidity at the adapted temperature. In this study, the homeoviscous adaptation in living cells was tested using a fluorescent probe, laurdan. As an index of membrane fluidity, we adopted the mean GP values at the anterior part of the cell, though GP values are widely distributed within the selected area. GP values measured at the culture temperature were similar irrespective of the actual temperature at which the cells had been cultured (Fig. 3), and the time for fluidity adaptation from 23°C to either 18°C or 28°C was about 1 h (Fig. 4). Therefore, the membrane fluidity is regulated at a constant level during adaptation to the environmental temperature.

The constant GP value at the culture temperature was greatly altered by slight changes of the measurement temperature from the culture temperature; in particular, when the measurement temperature was lower than the culture temperature, GP values increased far above the adapted level. Hence, the temperature-dependent fluidity change is largest at around the culture temperature, i.e. the adapted fluidity is adjusted at almost the midpoint of the temperature-dependent change in membrane fluidity. The temporal changes in fluidity are so fast as to immediately follow the temperature drop induced by the cooling stimulus (Fig. 3A); such temperature dependence raises the possibility that a rapid change in fluidity initiated from the adapted level causes the activation of cooling-sensitive Ca^{2+} channels. This would offer a good explanation for the cooling-induced depolarization, which was most sensitive at the culture temperature. Consistent with this possibility, chemical modulation of the adapted fluidity to either an increased or a

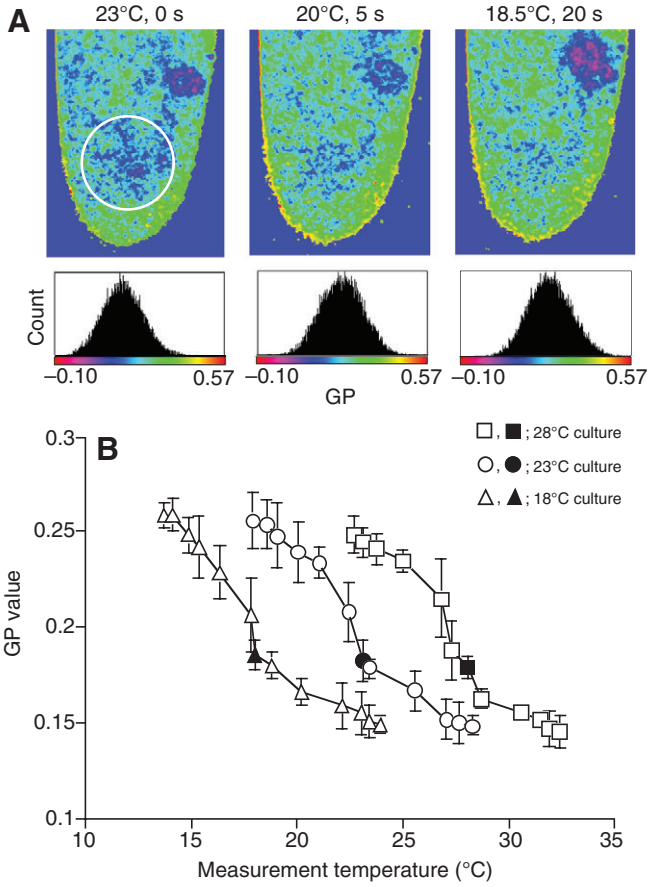

Fig. 3. Generalized polarization (GP) values measured at various temperatures. (A) GP images of the anterior cell and corresponding histograms of GP values. The GP value of each pixel was measured within the circle (diameter, 20 μm) and displayed as a histogram; the mean GP value was determined from the mean of the histogram. In this case, GP images were obtained from a single cell cultured at 23°C. The measurement temperature was initially controlled at 23°C for 1 min, then rapidly cooled to 18°C. The measurement temperature and the time after the start of cooling are indicated above the GP images. Note that the GP histogram shifts to larger values with the temperature drop. (B) Effect of culture temperature on GP values measured at various temperatures. Cells were cultured at 18°C, 23°C or 28°C and GP values were measured at various temperatures indicated on the abscissa. In order to avoid adaptation to a temperature different from the culture temperature, the series of temperature changes in the fluorescence imaging was carried out within a few minutes. Filled symbols indicate measurements at the respective culture temperatures. Each plot of GP value is the mean ± s.d. (*N*=5–8 cells).

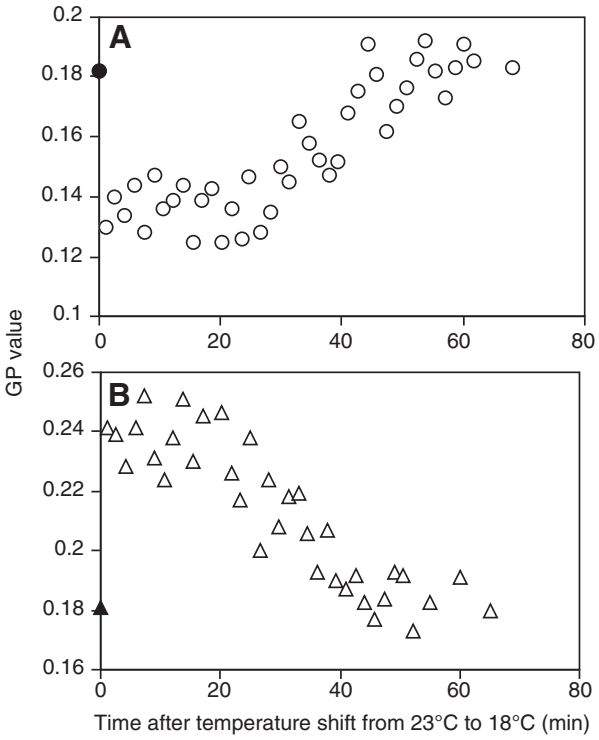

Fig. 4. Adaptation of GP value following a shift of the culture temperature. Cells cultured at 23°C were deciliated to stop cellular movements in fluorescence imaging and stained with laurdan. Cells were stained for 30 min at 23°C, and the temperature was then shifted to either 28°C (A) or 18°C (B) at time zero on the abscissa. Filled symbols show GP measurements at 23°C before the temperature shift. Open symbols show GP measurements at the shifted temperatures. GP values shown in A and B were taken from different cells.

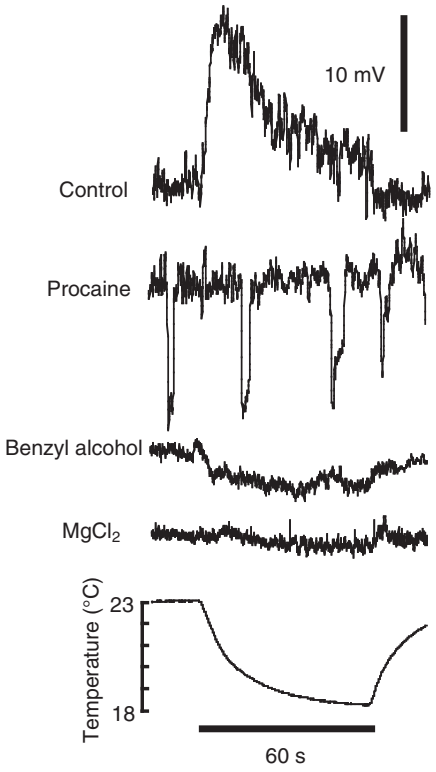

Fig. 5. Inhibition of cooling-induced depolarization. Chemicals were applied to cells adapted at 23°C and left for 5–10 min, then stimulated with a temperature drop from 23°C to 18°C as shown in the bottom trace. The transient depolarization elicited in the control condition disappeared following addition of 30 mmol l^{-1} procaine, 30 mmol l^{-1} benzyl alcohol or 8 mmol l^{-1} $MgCl_2$. Addition of procaine frequently caused hyperpolarizing oscillation, though the reason for this is unknown. Resting potential before the temperature drop was controlled at approximately –25 mV with a current clamp.

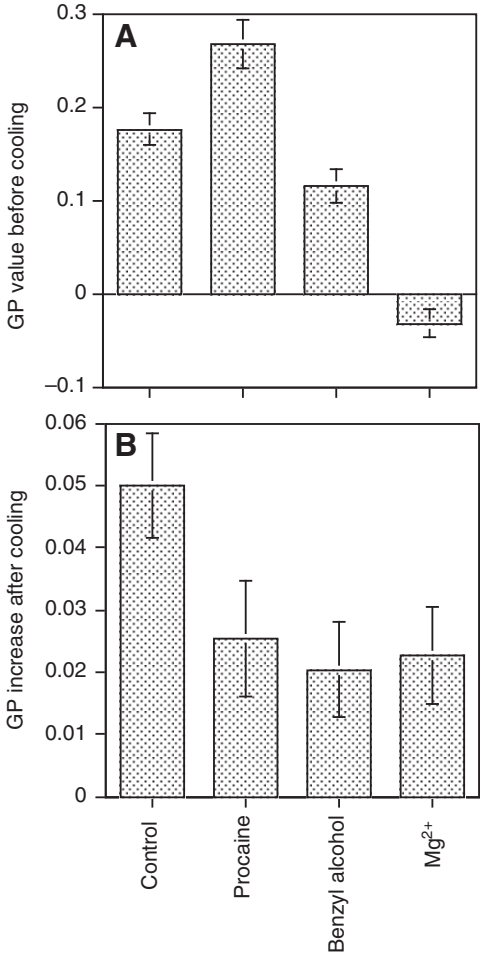

Fig. 6. Alteration of GP values by addition of chemicals. Cells cultured at 23°C were labelled with laurdan at 23°C and stimulated with a cooling stimulus of a temperature drop from 23°C to 18°C. (A) GP values measured at 23°C before the cooling stimulus. Compared with control, the GP value was increased by the addition of 30 mmol l^{-1} procaine, but was decreased by addition of either 30 mmol l^{-1} benzyl alcohol or 8 mmol l^{-1} $MgCl_2$. (B) Extent of GP increase after cooling. Cells measured in A were subjected to a temperature drop to 18°C, and GP values were measured 5 s later. Each GP value is the mean ± s.d. (*N*=3–6 experiments).

decreased level abolished the cooling-induced depolarization (Figs 5 and 6). Therefore, a constant fluidity at the adapted temperature and its quick decrease after cooling seems to be important for the activation of cooling-sensitive Ca^{2+} channels.

Membrane proteins including ionic channels interact with the surrounding lipid bilayer. In mechano-sensitive channels, channel activity is altered by mechanical tension applied by the surrounding membrane (Meyer et al., 2006; Wiggins and Phillips, 2005). Additionally, thermo-sensitive transient receptor potential channels (TRPM8) are activated by interactions with the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) (Rohács et al., 2005). For the cooling-sensitive channels of *Paramecium,* the fluidity decrease following the cooling stimulus will alter some interactions between membrane lipids and channels, which will result in activation of these channels.

Paramecium cells retain another type of thermo-sensitive channel, heating-sensitive channels, which are permeable to Ca^{2+} and are activated by heating above the culture temperature (Imada and Oosawa, 1999). Considering that the activation of these channels is also dependent on the culture temperature, the heating-sensitive channels may be activated by an increase of membrane fluidity above the adapted level, although this prediction was not examined in the present study.

In conclusion, *Paramecium* cells adapt to the environmental temperature by regulation of the membrane fluidity to a constant level. The constant level is greatly altered by small changes in the surrounding temperature. Such a temperature dependence of membrane fluidity raises the possibility that the fluidity change from the adapted level triggers the opening of cooling-sensitive channels. This would explain well the maximum response at the adapted temperature. A memory of the culture temperature remains in the cell membrane as a homeoviscous adaptation, which is reflected in the thermo-sensitive behaviour.

This work was supported, in part, by a grant from the Ministry of Sport, Culture, Science and Education of Japan (14580675).

REFERENCES

Anderson, R. L., Minton, K. W., Li, G. C. and Hahn, G. M. (1981). Temperature-induced homeoviscous adaptation of Chinese hamster ovary cells. *Biochim. Biophys. Acta* **641**, 334-348.

Chapman, C. F., Liu, Y., Sonek, G. J. and Tromberg, B. J. (1995). The use of exogenous fluorescent probes for temperature measurements in single living cells. *Photochem. Photobiol.* **62**, 416-425.

Chong, P. L. and Wong, P. T. (1993). Interactions of laurdan with phosphatidylcholine liposomes: a high pressure FTIR study. *Biochim. Biophys. Acta* **1149**, 260-266.
Harris, F. M., Smith, S. K. and Bell, J. D. (2001). Physical properties of erythrocyte ghosts that determine susceptibility to secretary phospholipase A_2. *J. Biol. Chem.* **276**, 22722-22731.
Hazel, J. R. (1995). Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* **57**, 19-42.
Hennessey, T. M. and Nelson, D. L. (1983). Biochemical studies of the excitable membrane of *Paramecium tetraurelia.* VIII. Temperature-induced changes in lipid composition and in thermal avoidance behavior. *Biochim. Biophys. Acta* **728**, 145-158.
Imada, T. and Oosawa, Y. (1999). Thermoreception of *Paramecium:* different Ca^{2+} channels were activated by heating and cooling. *J. Membr. Biol.* **168**, 283-287.
Jennings, H. (1906). *Behavior of Lower Organisms*, pp. 65-71. Bloomington, IN: Indiana University Press.
Kuriu, T., Nakaoka, Y. and Oosawa, Y. (1996). Cold-sensitive Ca^{2+} influx in *Paramecium. J. Membr. Biol.* **154**, 163-167.
Mamdouh, Z., Giocondi, M. C. and Le Grimellec, C. (1998). In situ determination of intracellular membrane physical state heterogeneity in renal epithelial cells using fluorescence ratio microscopy. *Eur. Biophys. J.* **27**, 341-351.
Martin, C. E., Hiramitsu, K., Kitajima, Y., Nozawa, Y., Skriver, L. and Thompson, Jr, G. A. (1976). Molecular control of membrane properties during temperature acclimation. Fatty acid desaturase regulation of membrane fluidity in acclimating *Tetrahymena* cells. *Biochemistry* **15**, 5218-5227.
McKinley, S. J. and Hazel, J. R. (2000). Does membrane fluidity contribute to thermal compensation of β-adrenergic signal transduction in isolated trout hepatocytes. *J. Exp. Biol.* **203**, 631-640.
Mendelssohn, M. (1895). Über den Thermotropismus ein-zelliger Organismen. *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **60**, 1-27.
Meyer, G. R., Gullingsrud, J., Schulten, K. and Martinac, B. (2006). Molecular dynamics study of MscL interactions with a curved lipid bilayer. *Biophys. J.* **91**, 1630-1637.
Nakaoka, Y. and Oosawa, F. (1977). Temperature sensitive behavior of *Paramecium caudatum., J. Protozool.* **24**, 575-580.
Nakaoka, Y., Kurotani, T. and Itoh, H. (1987). Ionic mechanism of thermoreception in *Paramecium. J. Exp. Biol.* **127**, 95-103.
Nakaoka, Y., Tokioka, R., Shinozawa, T., Fujita, J. and Usukura, J. (1991). Photoreception of *Paramecium* cilia: localization of photosensitivity and binding with anti-frog-rhodopsin IgG. *J. Cell Sci.* **99**, 67-72.
Oosawa, F. and Nakaoka, Y. (1977). Behavior of microorganism as particles with internal state variables. *J. Theor. Biol.* **66**, 747-761.
Palleschi, S. and Silvestroni, L. (1996). Laurdan fluorescence spectroscopy reveals a single liquid-crystalline lipid phase and lack of thermotropic phase transitions in the plasma membrane of living human sperm. *Biochim. Biophys. Acta* **1279**, 197-202.
Parasassi, T., De Stasio, G., d'Ubaldo, A. and Gratton, E. (1990). Phase fluctuation in phospholipid membranes revealed by laurdan fluorescence. *Biophys. J.* **57**, 1179-1186.
Parasassi, T., De Stasio, G., Ravagnan, G., Rusch, R. M. and Gratton, E. (1991). Quantitation of lipid phases in phospholipid vesicles by the generalized polarization of laurdan fluorescence. *Biophys. J.* **60**, 179-189.
Rohács, T., Lopes, C. M., Michailidis, I. and Logothetis, D. E. (2005). $PI(4,5)P_2$ regulates the activation and desensitization of TRPM8 channels through TRP domain. *Nat. Neurosci.* **8**, 626-634.
Sasaki, T., Konoha, Y., Toyoda, T., Yasaka, Y., Przybos, E. and Nakaoka, Y. (2006). Correlation between thermotolerance and membrane properties in *Paramecium aurelia. J. Exp. Biol.* **209.** 3580-3586.
Sheffield, M. J., Baker, B. L., Li, D., Owen, N. L., Baker, M. L. and Bell, J. D. (1995). Enhancement of Agkistrodon piscivorus venom phospholipase A_2 activity toward phosphatidylcholine vesicles by lysolecithin and palmitic acid: studies with fluorescent probes of membrane structure. *Biochemistry* **34**, 7796-7806.
Sinensky, M. (1974). Homeoviscous adaptation-a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli. Proc. Nat. Acad. Sci. USA* **71**, 522-525.
Soicic, Z., Toplak, H., Zuehlke, R., Honegger, U. E., Buhlmann, R. and Wiesmann, U. N. (1992). Cultured human skin fibroblasts modify their plasma membrane lipid composition and fluidity according to growth temperature suggesting homeoviscous adaptation at hypothermic (30°C) but not at hyperthermic (40°C) temperatures. *Biochim. Biophys. Acta* **1104**, 31-37.
Tominaga, T. and Naitoh, Y. (1994). Comparison between thermoreception and mechanoreceptor currents in *Paramecium caudatum. J. Exp. Biol.* **189**, 117-131.
Vest, R. S., Gonzales, L. J., Permann, S. A., Spencer, E., Hansen, L. D., Judd, A. M. and Bell, J. D. (2004). Divalent cations increase lipid order in erythrocytes and susceptibility to secretory phospholipase A_2. *Biophys. J.* **86**, 2251-2260.
Wiggins, P. and Phillips, R. (2005). Membrane-protein interactions in mechanosensitive channels. *Biophys. J.* **88**, 880-902.
Williams, E. and Somero, G. (1996). Seasonal-, tidal-cycle- and microhabitat-related variation of phospholipid vesicles from gills of the intertidal mussel *Mytilus calfornianus. J. Exp. Biol.* **199**, 1587-1596.
Yu, W., So, P. T., French, T. and Gratton, E. (1996). Fluorescence generalized polarization of cell membranes: a two-photon scanning microscopy approach. *Biophys. J.* **70**, 626-636.