Biochimica et Biophysica Acta, 728 (1983) 145–158
Elsevier Biomedical Press

BBA 71528

BIOCHEMICAL STUDIES OF THE EXCITABLE MEMBRANE OF *PARAMECIUM TETRAURELIA*

VIII. TEMPERATURE-INDUCED CHANGES IN LIPID COMPOSITION AND IN THERMAL AVOIDANCE BEHAVIOR

TODD M. HENNESSEY and DAVID L. NELSON

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706 (U.S.A.)

(Received July 29th, 1982)

Key words: Thermotaxis; Avoidance reaction; Homeoviscous adaptation; Membrane lipid; Lipid composition

Transfer of a culture of *Paramecium tetraurelia* from 25 to 35°C produced several significant changes in membrane lipid composition within 4 h. The proportion of gg-linolenic acid (γ18:3) decreased, the ratio of 16:0 to 18:0 in sphingolipids decreased, and the ratio of 7-dehydrocholesterol to 7-dehydrostigmasterol increased at the higher temperature. When cultures were transferred from 35 back to 25°C, these changes were reversed within 4 h. The lipid alterations induced by a temperature shift occurred both in ciliary membranes and in deciliated bodies, but were most striking in ciliary membranes. The shift up in culture temperature caused a slight reduction in the proportion of ciliary unsaturated fatty acids within 8 h. However, after two days at 35°C the proportion of unsaturated fatty acids of ciliary membranes was not different from that of ciliary membranes from cells cultured at 25°C. Coincident with the changes in membrane lipid composition, thermal avoidance behavior of paramecia also changed after a shift from 25 to 35°C. Cells incubated at the higher temperature showed less thermal avoidance, but reacquired it after being transferred back to 25°C. The changes in thermal avoidance behavior occur in parallel with changes in membrane lipid composition, suggesting that the membrane lipid may be involved in thermosensory transduction.

Introduction

Changes in growth temperature have been shown to cause alterations in the membrane lipid composition of cells ranging from brain to bacteria [1–5]. Thermal acclimatization of membrane lipids has been especially well studied in the ciliated protozoan *Tetrahymena pyriformis* [6]. Similar changes in membrane lipid composition can be produced isothermally in many organisms either by the addition of agents that perturb membrane fluidity [7–11] or by lipid supplementation [12–18]. These data indicate that the lipid changes are due to a temperature-induced change in membrane fluidity and not to some other effect of temperature. The lipid changes are compensatory; 'fluidizing' agents or conditions are counteracted by changes in lipid composition which make the membrane less fluid, and 'solidifying' conditions cause lipid composition changes which increase the membrane's fluidity. This acclimatization response of membrane lipids is referred to as 'homeoviscous adaptation' [2].

Since the activities of many membrane-bound enzymes are dependent upon associated lipid composition and fluidity [19–24], the regulation of

Abbreviations: Mops, 4-morpholinepropanesulfonic acid; DPH, diphenylhexatriene.

membrane lipid composition may be involved in maintaining the proper environment for membrane protein activity. Alterations in lipid composition might therefore alter membrane function. Environmentally-induced lipid changes have been correlated with changes in a variety of cellular functions such as chemotactic behavior in *Tetrahymena* [25], thermotaxis in *Dictyostelium* (Whitaker, B., (1979) Ph.D. thesis, Michigan State Univ.) and resistance to high temperature in Chinese hamster ovary cells [5].

Our interests center upon the function of the surface membrane of *Paramecium.* In this unicellular eucaryote, voltage-sensitive Ca^{2+} channels in the ciliary membrane open in response to a variety of depolarizing stimuli. The resulting influx of Ca^{2+} triggers a change in the direction of the ciliary beat, and the protozoan swims backward, away from the stimulus. We have shown [26] that *Paramecium* avoids a warm region by this means, and that the exact temperature at which thermal avoidance occurs depends upon the temperature at which cells were previously cultured. We have postulated that heat-induced changes in the structure of the lipid bilayer might affect the function of membrane proteins embedded in that bilayer and that the lipids of the ciliary or cell body membrane might actually be the primary thermosensor in *Paramecium.* Recently we found [27] that certain mutants of *Paramecium* with clear alterations in excitable membrane function also have altered membrane lipids. This finding suggests that membrane lipids may also be involved in excitability. To test this hypothesis further, we have examined the effect of growth temperature upon membrane lipid composition and upon thermal avoidance behavior in *Paramecium.*

Materials and Methods

Cell growth, harvesting and deciliation

Paramecium tetraurelia (stock 51s) were grown axenically at 25 or 35 ± 1.0°C in Soldo's crude medium [28]. In the temperature shift experiments mid-log phase cultures grown at 25°C were transferred to 35°C. The temperature of the culture increased at about 7°C/h until the final temperature was reached (about 2 h).

Cells were harvested, washed in Dryl's solution (1 mM NaH_2PO_4/1 mM Na_2HPO_4/2 mM trisodium citrate/1.5 mM $CaCl_2$, pH 6.8), and deciliated on ice as described by Hennessey et al. [29]. Whole cell, body or cilia samples were frozen in dry ice-acetone and stored at −20°C under N_2.

Sterol and fatty acid analysis

Whole cell, body, and ciliary lipids were extracted by a modification of the Bligh and Dyer method [30] as described by Andrews and Nelson [31]. Butylated hydroxytoluene (final concentration of 5 μg/ml) was added as an antioxidant. All solvents were redistilled before use.

Neutral lipids were separated from phospholipids by silicic acid column chromatography [32] for analysis by gas chromatography. The sterols in the neutral lipid fraction were analyzed with a Varian gas chromatograph (model 3700) interfaced with a CDS 111 integrator. The ion detector temperature was 350°C and injector temperature was 320°C. A 3 ft. glass column packed with 3% OV-17 on 100/120 mesh Supelcoport (Supelco) was heated from 260 to 330°C at 5°C/min to elute the sterols. The carrier gas was N_2 at a flow rate of 20 cc/min. Identification and quantitation of the sterols was determined by co-chromatography with known amounts of cholesterol, 7-dehydrocholesterol, and stigmasterol (Sigma).

The acyl- and amide-linked fatty acids and glyceryl ethers of the phospholipid fraction were also analyzed by gas chromatography. Fatty acids were sequentially removed from the phospholipid fraction. The acyl-linked fatty acids were first removed by mild alkaline hydrolysis in 0.6 N methanolic NaOH at room temperature for 1 h. The fatty acid methyl esters generated by this procedure were separated from the mild alkaline-stable phospholipids by silicic acid column chromatography (fatty acid methyl esters elute in the neutral lipid fraction). The amide-linked fatty acids of sphingolipids were then removed by strong acid hydrolysis of the remaining phospholipid fraction in 5.0 ml HCl/H_2O/MeOH (4.3 : 4.7 : 41) at 70°C overnight. The fatty acid methyl esters were extracted with 3 vol. hexane. All of these methods are described by Kates [32].

The glyceryl ethers were generated from the remaining lysolipids by addition of the Vitride reagent as described by Rhoads and Kaneshiro

[33]. Ether-containing lipids were removed from the aqueous phase (5 ml) of the previous reaction mixture by adding about 6 ml $CHCl_3$ and 3 ml H_2O, vortexing, centrifuging, and removing the lower ($CHCl_3$) phase. The $CHCl_3$ phase was dried and the lipids reduced by adding the Vitride reagent (J.T. Baker Chemical Co.). This procedure removed the phospholipid head groups, leaving the glyceryl ethers. These were derivatized by adding 50 μl of trimethylsilyl reagent (Sil-Prep, Applied Science Laboratories) and incubating at 37°C for 1 h. Identification was based on co-chromatography with standards by gas chromatography as described by Rhoads et al. [34].

The fatty acid methyl esters from mild alkaline hydrolysis and strong acid hydrolysis and derivatized glyceryl ethers were analyzed by gas chromatography. A 6 ft. column packed with 10% SP-2330 on 80/100 mesh Supelcoport (Supelco) was temperature programmed from 150 to 200°C at 6°C/min. Injector temperature was 270°C, ion detector temperature was 300°C and the N_2 carrier gas had a flow rate of about 20 cc/min. Quantitation of the fatty acid methyl esters was done by comparison to a known amount of 16:0 methyl ester and identification was based on co-chromatography with fatty acid methyl ester standards (Supelco). Analysis of the glyceryl ethers was done by coelution with known amounts of derivatized chimyl and batyl alcohol (Sigma).

Phospholipid analysis

Cells were labeled with $^{32}P_i$ and analyzed by two-dimensional thin layer chromatography as described by Rhoads and Kaneshiro [33]. Cultures were supplemented with 100 μCi/l [^{32}P]H_3PO_4 (New England Nuclear) for 5 generations. The extracted ciliary lipid fraction was spotted on 250 μm thin layer chromatography plates (Silica gel 60, Scientific Products) and chromatographed in the first dimension with $CHCl_3$/MeOH/NH_4OH (65 : 25 : 5) and in the second dimension in $CHCl_3$/acetone/MeOH/acetic acid/H_2O (30 : 40 : 10 : 10 : 5). Spots were identified by I_2 vapor and autoradiography, scraped and counted as described by Andrews and Nelson [31]. Phospholipid concentration was determined by the method of Chen et al. [35].

Behavioral analysis

Thermal avoidance of 42°C in 'control solution' (1 mM Mops/1 mM $Ca(OH)_2$/0.35 mM citrate, pH 7.0) was measured as described by Hennessey and Nelson [26]. An increase in the I_T (index of thermal avoidance) value represents a decrease in thermal avoidance of 42°C; I_T ranges from 1.0 (no thermal avoidance) to 0.0 (complete avoidance of the test temperature). The I_m (index of motility) value is a gross measure of motility [26]. I_m ranged from 0.10 to 0.30; a larger I_m represents an increased motility. During these tests the cells were at room temperature (23 ± 2.0°C) for 5–10 min.

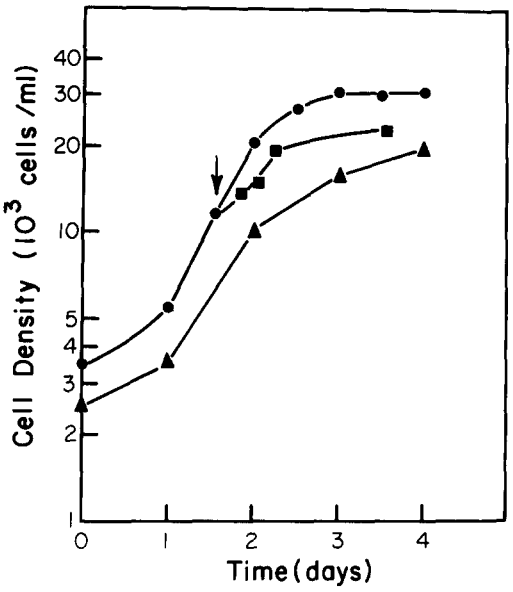
Results

Thermal avoidance behavior

Cultures grown at 25°C for two doublings and shifted to 35°C showed a decrease in final cell yield as did those grown for three doublings at 35°C (Fig. 1).

Cells incubated at 35°C for 4 h or more lost the ability to avoid 42°C (as shown by an increase in I_T) but regained thermal avoidance after 4 h at 25°C (Fig. 2). Transfer of cultures from 25 to 35°C produced an increase in cell motility (I_m increased from 0.14 ± 0.01 (25°C) to 0.19 ± 0.02 (35°C) after 8 h). This increase in I_m was maintained in cultures returned to 25°C for 4 h. Even 2 days after transfer to 35°C the I_T and I_m measurements were higher than for cells incubated at 25°C (for 25°C $I_T = 0.62 \pm 0.07$, $I_m = 0.06 \pm 0.0$ and for 35°C $I_T = 0.91 \pm 0.01$, $I_m = 0.14 \pm 0.03$).

Fatty acids of the phospholipids of whole cells. Although the fatty acid composition of whole cells grown at 35°C was qualitatively similar to that of cells grown at 25°C, there were differences in the proportions of several specific fatty acids that were reproducible in two separate experiments. Among the acyl-linked fatty acids of cells grown at 35°C, there was a clear decrease in γ18 : 3 (from about 21% to about 15%), a slight decrease in 20 : 4 and a slight increase in 16 : 0, as compared with those of cells grown at 25°C. The result of these changes was an apparent reduction in unsaturation, measured either as percent unsaturated fatty acids, or by the index of unsaturation (Table IA). In the amide-linked (sphingolipid) fraction of fatty acids, the principle components were 16 : 0, 17 : 0 and



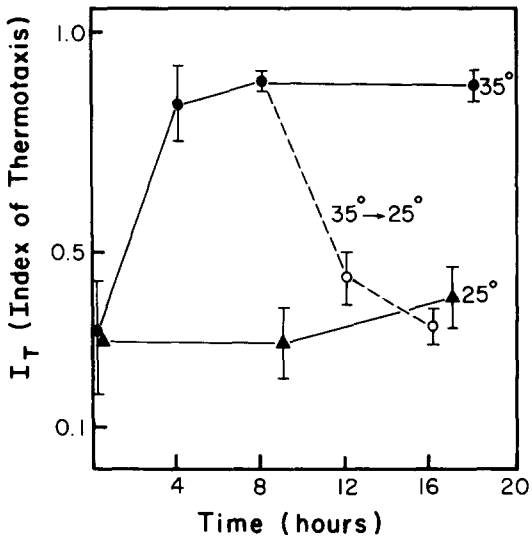


Fig. 1. Growth of axenic *Paramecium* at 35 and 25°C. Cells were grown at 25°C for two generations and then either kept at 25°C (●———●) or transferred to a 35°C incubator (■———■). The arrow shows the cell density at the time of transfer to 35°C. Cells were also grown for 3 generations at 35°C (▲———▲). Each point represents one culture but similar growth curves were obtained in other experiments.

Fig. 2. Thermal avoidance of 42°C as a function of time at 25° and 35°C. Cells were washed and tested in 'control solution' (1 mM Mops/1 mM $Ca(OH)_2$/0.35 mM citrate, pH 7.0). The I_T represents the percentage of cells entering the heated arm of a three-way stopcock; as thermal avoidance decreased I_T increased. Cells were grown for two generations at 25°C and then either kept at 25°C (▲———▲) or transferred to 35°C (●———●). After 8 h at 35°C some cells were returned to 25°C (○——— ○). ($n = 2$).

18 : 0. Growth at 35°C resulted in a 3-fold reduction in the ratio of 16 : 0 to 18 : 0 (Table IB). Acyl-linked fatty acids represent 95–98% of the total fatty acids in phospholipids of whole cells. Thus, the changes in whole cell phospholipids produced by incubation at 35°C were essentially those of the acyl-linked fatty acids, with an overall decrease in unsaturation (Table IC).

The changes in whole-cell fatty acid composition began within 4 h of the shift from 25 to 35°C, and were complete within 8 h (Table I). These changes were partially or wholly reversible upon transfer back to 25°C. By 12 h after the transfer the percentage of γ18 : 3 and the relative unsaturation were indistinguishable from those of cells cultured continuously at 25°C. Also, the ratio of 16 : 0 to 18 : 0 in amide-linked (sphingolipid) fatty acids approached that of cells cultured at 25°C (Table IB).

Fatty acids of the phospholipids of bodies and cilia. To determine whether the compositional changes seen in whole cells are localized in bodies or cilia, we examined each fraction separately with cells cultured at 25°C or shifted to 35°C (Tables II and III). Deciliated bodies contain 80–90% of the phospholipids of whole cells [31], and as expected, the fatty acids of phospholipids from bodies represented about 93% of the total phospholipid-bound fatty acids. A very small proportion (2–6%) of the fatty acids of body phospholipids were amide-linked confirming the reports that the sphingolipids of *Paramecium* are concentrated in cilia [31,33]. The principle fatty acids of body phospholipids were 16 : 0, 18 : 1, 18 : 2, γ18 : 3 and 20 : 4.

The changes in fatty acid composition of body phospholipids after a shift in growth temperature from 25 to 35°C were similar to those seen in whole cells (Table II); the relative unsaturation and unsaturation index decreased within 4 h at 35°C, and remained lower than 25°C controls for

TABLE I

WHOLE CELL FATTY ACIDS FROM PHOSPHOLIPIDS AS A FUNCTION OF TIME AT 25 AND 35°C

Cells were grown at 25°C for two generations and then either kept at 25°C or transferred to 35°C. Cells for the 35 → 25°C sample were at 35°C for 12 h and returned to 25°C for an additional 12 h. All entries are expressed as percent of total (acyl + amide-linked) fatty acids of the phospholipid fraction. The unsaturation index (U.I.) = the summation of the percent of total times the number of double bonds for each unsaturated fatty acid. Data are shown for acyl-linked (A), amide-linked (B), and total (C) fatty acids. The data represent the results of a typical experiment.

Fatty acid	25°C				35°C			35 → 25°C
	4 h	8 h	12 h	24 h	4 h	8 h	12 h	12 h
Acyl-linked (A)								
14:0	1.1	1.0	1.1	0.92	1.5	1.3	1.4	0.82
15:0	0.58	0.53	0.57	0.63	0.68	0.58	0.62	0.58
16:0	19.7	18.2	17.5	16.6	22.3	20.1	19.6	16.3
16:1	1.6	1.5	1.6	1.7	1.7	1.5	1.8	1.4
17:0	0.07	0.08	0.09	0.08	0.10	0.11	0.06	0.07
18:0	0.48	0.48	0.55	0.47	0.64	0.57	0.42	0.47
18:1	13.4	13.4	13.8	11.7	16.5	15.7	14.1	11.3
18:2	16.4	17.0	16.5	19.7	15.3	21.2	22.0	22.8
γ18:3	21.1	20.7	20.8	19.4	16.9	14.0	14.6	18.6
20:1	1.0	0.77	0.82	1.2	1.0	0.89	1.4	0.90
20:3	1.0	1.3	1.1	1.5	0.93	1.2	1.3	1.1
20:4	20.3	21.3	21.9	21.8	19.0	19.5	18.4	21.3
20:5	0.86	1.3	1.0	0.41	0.86	0.87	1.2	1.1
% unsat.	77.5	79.3	79.5	80.7	74.1	76.9	77.0	81.2
U.I.	202.7	209.3	206.5	213.4	185.81	189.2	188.3	212.7
Amide-linked (B)								
16:0	1.9	2.2	2.3	3.4	1.9	1.9	2.3	2.7
17:0	0.09	0.09	0.12	0.15	0.09	0.07	0.27	1.4
18:0	0.34	0.24	0.25	0.33	0.72	0.72	1.3	0.57
16:0/18:0	5.6	9.2	9.2	10.3	2.6	2.6	1.8	4.7
Total (C)								
Total % unsat.	75.7	77.3	77.5	77.5	72.2	74.9	74.8	78.5
Total U.I.	204.1	207.4	208.0	205.6	183.6	188.4	188.6	209.0
% amide	2.3	2.5	2.7	3.9	2.7	2.8	3.9	4.7

as long as the culture was maintained at 35°C. When cultures were shifted back to 25°C, this change in unsaturation was reversed within 4 h. These temperature-induced changes in unsaturation were due primarily to a reproducible decrease in γ18 : 3 at 35°C, and the subsequent increase in γ18 : 3 towards the initial (25°C) level when cells were shifted back to 25°C (Fig. 3). The sphingolipid fatty acids of deciliated bodies changed little after the shift in temperature. In particular, the decrease in 16 : 0 to 18 : 0 ratio which occurred in whole cells was not observed in bodies (Table IIB).

The fatty acid composition of phospholipids from isolated cilia was strikingly different from that of body phospholipids (compare Tables II and III); 20 : 4 was much more prominent in cilia, as were the amide-linked fatty acids of sphingolipids 16 : 0, 17 : 0 and 18 : 0.

When cells were shifted from 25 to 35°C, the most significant change in ciliary fatty acid composition occurred in their sphingolipid fraction. The ratio of 16 : 0 to 18 : 0 in this fraction decreased within 4 h, and by 18 h this ratio was 3.6-times lower in the cells at 35 than in 25°C controls. This change in sphingolipid fatty acids was partially reversed within 8 h after 35°C cultures were returned to 25°C (Fig. 4).

The proportion of γ18 : 3 changed significantly

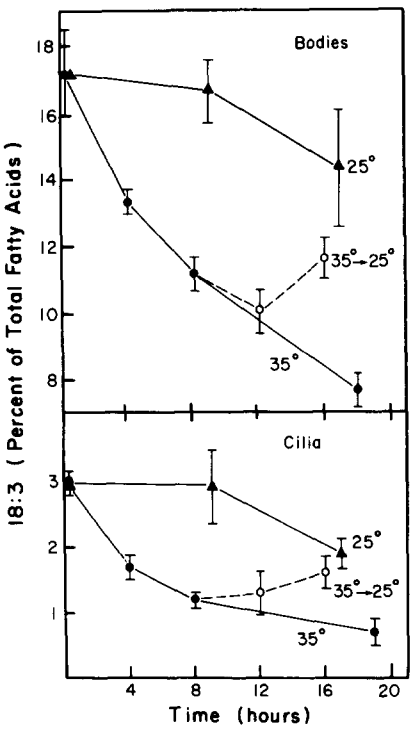


Fig. 3. Amount of γ18:3 of body or cilia, expressed as percent of total fatty acids, as a function of time at 25 and 35°C. Cells were grown for two generations at 25°C and then either kept at 25°C (▲———▲) or transferred to 35°C (●———●). After 8 h at 35°C some cells were returned to 25°C (○———○). Cells from each time point were deciliated and the total fatty acids extracted and analyzed by gas chromatography as described in Materials and Methods. ($n = 2$).

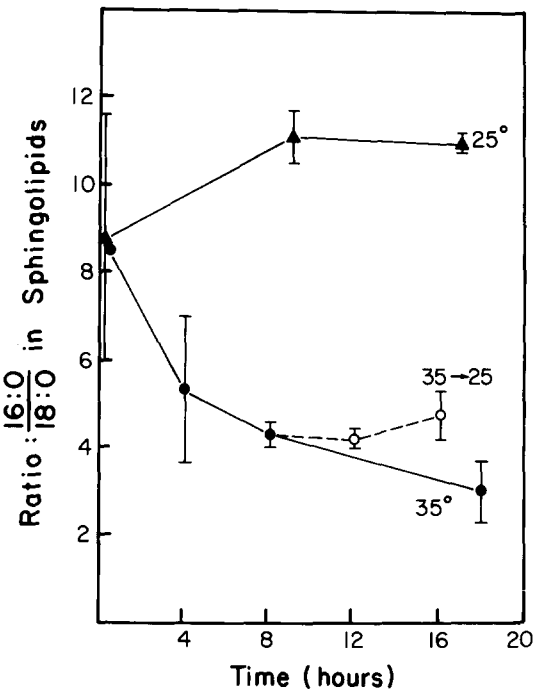


Fig. 4. Ciliary sphingolipid 16:0/18:0 as a function of time at 25 and 35°C. Cells were grown for two generations at 25°C and then either kept at 25°C (▲———▲) or transferred to 35°C (●———●). After 8 h at 35°C some cells were returned to 25°C (○———○). Cells from each time point were deciliated and the amide-linked fatty acids of the cilia were analyzed by gas chromatography as described in Materials and Methods ($n = 2$).

in the acyl-linked fraction of ciliary phospholipids after the shift to 35°C. Smaller changes in 18:1, 18:2 and 20:4 were not statistically significant ($P > 0.1$ by Student's t test). The decrease in ciliary γ18:3 was reversed when cultures were returned to 25°C (Fig. 3).

Although the fatty acid composition of body and cilia differed, the total index of unsaturation for ciliary phospholipids was very nearly the same as that for body phospholipids (compare Tables IIC and IIIC). The shift from 25 to 35°C resulted in an increase in the percent of ciliary amide-linked fatty acids within 4 h, and in a consequent reduction in the total unsaturation. However, this effect was transient; 18 h after the temperature shift, the difference in unsaturation and percent of amide-linked fatty acids was not significant (Table IIIC).

Sterols of bodies and cilia. Axenically-cultured paramecia contain two principle sterols: 7-dehydrocholesterol and 7-dehydrostigmasterol [29,36]. To determine whether the kind or amount of sterol in membranes might change in response to the shift to 35°C, we extracted and measured the sterols of bodies and cilia. Although the ratios of sterol to phospholipid in bodies and cilia were unchanged after the temperature shift, the ratio of 7-dehydrocholesterol to 7-dehydrostigmasterol did increase within 4 h at 35°C, and decreased to the level in 25°C control cells when the culture was returned to 25°C (Fig. 5). The change occurred in both bodies and cilia, but was more striking in cilia, which are relatively enriched for sterols.

TABLE II

BODY FATTY ACIDS FROM PHOSPHOLIPIDS AS A FUNCTION OF TIME AT 25 AND 35°C

Cells were grown for two generations and then either kept at 25°C, transferred to 35°C or returned from 35 to 25°C. At each time point the cells were deciliated and extracted as described in Materials and Methods. Cells returned to 25°C (35 → 25°C) were at 35°C for 8 h. The percent of total fatty acids are shown for acyl (A), amide (B), and total (C) fatty acids. ($n = 2$).

Fatty acids	25°C			35°C			35 → 25°C	
	0 h	9 h	17 h	4 h	8 h	18 h	4 h	8 h
Acyl-linked (A)								
14:0	0.68	0.79	0.77	0.80	0.80	1.0	0.53	0.69
15:0	0.32	0.31	0.29	0.28	0.27	0.35	0.20	0.29
16:0	20.6	19.6	14.9	21.4	20.1	26.3	20.3	16.2
16:1	1.6	1.3	1.3	1.6	1.2	1.1	0.93	1.2
17:0	0.53	0.06	0.24	0.07	0.13	0.33	0.53	0.27
18:0	1.5	1.9	1.2	3.1	2.1	1.5	1.6	1.4
18:1	15.1	14.3	12.8	18.8	20.1	17.0	17.1	15.1
18:2	13.7	15.0	22.2	13.7	13.5	22.3	20.7	20.6
γ18:3	15.5	14.8	15.2	12.7	10.0	7.2	10.0	11.5
20:1	1.2	1.2	1.4	1.2	1.1	1.2	1.0	1.3
20:3	1.3	1.4	2.2	1.3	2.0	3.6	3.5	2.6
20:4	18.9	18.0	16.4	17.0	16.4	14.6	19.2	17.5
20:5	6.7	6.0	5.6	6.1	5.8	4.8	6.1	5.8
% unsat.	75.8	76.0	81.6	73.9	74.9	76.6	83.9	80.0
U.I.	210.0	208.2	217.7	193.5	192.2	190.6	222.3	211.5
Amide-linked (B)								
16:0	1.2	3.1	3.9	1.4	3.4	3.7	3.0	3.4
17:0	0.54	0.12	0.52	0.07	0.23	0.74	1.1	0.59
18:0	0.69	2.0	1.1	0.72	2.8	1.7	2.2	1.7
16:0/18:0	1.7	1.6	3.5	1.9	1.2	2.2	1.4	2.0
Total (C)								
Total % unsat.	74.0	72.1	77.1	72.3	70.1	71.8	78.6	75.5
Total U.I.	204.8	197.4	199.1	189.5	180.0	178.7	208.2	200.1
% amide	2.4	5.2	5.5	2.2	6.5	6.2	6.3	5.6

Long-term effects of growth at high temperature

Fatty acids of body phospholipids. After 2 days at 35°C, cells had undergone one division at the high temperature. The phospholipids of deciliated bodies showed the same reduction in polyunsaturated fatty acids (γ18:3, 18:2, 20:4) which had been seen after just 8 h at 35°C (compare Tables II and IV). Several further changes had occurred in the deciliated body fraction during the longer period at 35°C: (1) the proportion of 16:0 in acyl linkage was lower than in 25°C controls, (2) the proportions of acyl-linked 20:3 and 18:1 increased 2- to 3-fold, (3) the ratio of 16:0 to 18:0 in the sphingolipid fraction was 3-times lower in cells grown at 35°C than in 25°C control cells and (4) the percentage of unsaturated fatty acids was higher in 35°C cells than in 25°C controls while the indices of unsaturation were very similar for both populations (Table IV).

Fatty acids of ciliary phospholipids. The cilia of cells grown at 35°C for 2 days showed fatty acid changes similar to those seen in deciliated bodies. The proportions of acyl-linked 16:0, 18:2 and γ18:3 were reduced and the proportions of amide-linked 18:0 and acyl-linked 20:1 were increased, relative to 25°C controls. The amount of 20:4 in ciliary phospholipids was the same for cells grown at both temperatures, and the 35°C

TABLE III

CILIA FATTY ACIDS FROM PHOSPHOLIPIDS AS A FUNCTION OF TIME AT 25 AND 35°C

Cells were grown for two generations and then either kept at 25°C, transferred to 35°C or returned from 35 to 25°C. At each time point the cells were deciliated and extracted as described in Materials and Methods. Cells returned to 25°C (35 → 25°C) were at 35°C for 8 h. The percent of total fatty acids are shown for acyl (A), amide (B) and total (C) fatty acids. ($n = 2$).

Fatty acid	25°C			35°C			35 → 25°C	
	0 h	9 h	17 h	4 h	8 h	18 h	4 h	8 h
Acyl-linked (A)								
14:0	0.35	0.30	0.30	0.29	0.30	0.28	0.25	0.28
15:0	0.20	0.17	0.18	0.17	0.16	0.20	0.14	0.16
16:0	8.7	7.2	7.4	8.2	7.3	7.1	7.0	7.5
16:1	1.3	1.2	0.70	0.89	1.6	1.1	1.2	0.73
18:0	0.37	0.31	0.31	0.52	0.44	0.40	0.33	0.33
18:1	5.7	5.7	4.2	6.0	5.8	6.3	6.7	5.9
18:2	3.6	4.9	4.5	2.7	2.8	3.7	4.5	4.3
γ18:3	3.0	2.8	1.9	1.7	1.2	0.74	1.3	1.6
20:1	0.48	0.68	0.49	0.37	0.77	0.61	1.1	0.55
20:3	0.27	0.64	0.35	0.31	0.56	0.70	0.78	0.55
20:4	33.9	36.9	36.5	30.9	36.2	36.3	39.9	36.5
20:5	10.4	11.3	11.8	9.0	11.0	10.0	12.8	11.0
% unsat.	85.8	88.9	88.3	85.2	88.1	88.3	90.0	88.1
U.I.	300.4	310.5	325.0	301.0	296.8	310.0	314.6	314.9
Amide-linked (B)								
16:0	27.3	24.6	27.6	30.9	24.8	22.8	18.4	24.1
17:0	1.3	1.2	1.6	2.4	1.3	2.0	1.3	1.6
18:0	3.5	2.3	2.6	6.1	5.8	8.1	4.4	5.2
16:0/18:0	8.7	10.0	10.8	5.3	4.3	3.0	4.3	4.7
Total (C)								
Total % unsat.	58.7	64.1	60.4	51.9	59.9	59.4	68.3	61.1
Total U.I.	212.1	231.8	226.1	187.3	218.9	214.9	247.8	223.2
% amide	31.9	28.1	31.7	39.4	31.9	32.8	24.1	30.8

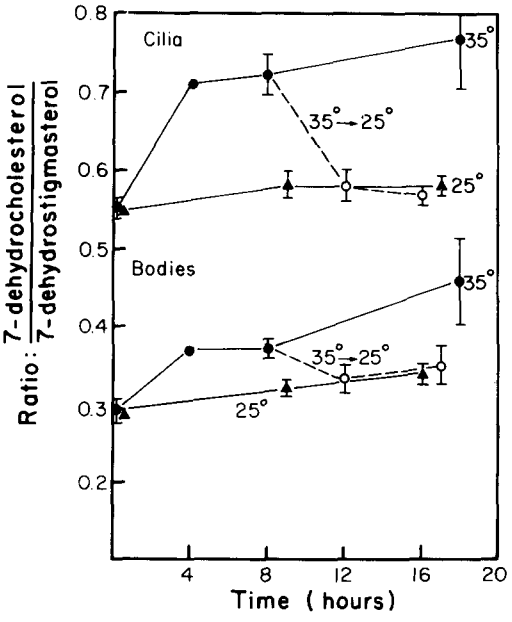


Fig. 5. Ratio of 7-dehydrocholesterol/7-dehydrostigmasterol for body and cilia as a function of time at 25 and 35°C. Cells were grown for two generations at 25°C and then either kept at 25°C (▲———▲) or transferred to 35°C (●———●). After 8 h some cells were returned to 25°C (○———○). Sterols were analyzed by gas chromatography as described in Materials and Methods. These 2 sterols represented greater than 90% of the sterols of body and cilia. ($n = 2$).

TABLE IV

BODY FATTY ACIDS FROM PHOSPHOLIPIDS AFTER 2 DAYS AT 25 AND 35°C

Cells were grown for two generations at 25°C and then either kept at 25°C or transferred to 35°C for 2 days, at which time the cells were deciliated and extracted as described in Materials and Methods. The percent of total fatty acids are shown for acyl (A), amide (B), and total (C) fatty acids. The numbers represent means ± standard deviation ($n = 2$).

Fatty acid	25°C	35°C
Acyl-linked (A)		
14:0	0.69 ± 0.19	0.56 ± 0.005
15:0	0.41 ± 0.08	0.18 ± 0.005
16:0	12.4 ± 0.70	7.3 ± 0.20
16:1	1.0 ± 0.08	0.73 ± 0.06
17:0	0.07 ± 0.0	0.05 ± 0.0
18:0	0.56 ± 0.02	0.61 ± 0.01
18:1	12.5 ± 0.35	22.5 ± 0.20
18:2	36.3 ± 0.4	32.9 ± 0.95
γ18:3	7.8 ± 0.10	4.3 ± 0.0
20:1	1.8 ± 0.20	2.1 ± 0.05
20:3	2.5 ± 0.50	7.4 ± 0.45
20:4	16.7 ± 0.25	13.5 ± 0.60
20:5	4.6 ± 0.25	4.4 ± 0.10
% unsat.	85.5 ± 1.0	91.1 ± 0.25
U.I.	210.8 ± 3.3	206.3 ± 1.7
Amide-linked (B)		
16:0	2.1 ± 0.15	1.5 ± 0.20
17:0	0.13 ± 0.03	0.19 ± 0.03
18:0	0.78 ± 0.005	1.6 ± 0.40
16:0/18:0	2.7 ± 0.21	0.97 ± 0.12
Total (C)		
Total % unsat.	83.0 ± 0.80	88.0 ± 0.90
Total U.I.	204.7 ± 2.9	199.6 ± 0.15
% Amide	2.9 ± 0.20	3.3 ± 0.65

cells contained somewhat more 20 : 5 than controls (Table VA).

Cilia contain significant amounts of 1-alkyl, 2-acyl phopsholipids [31,33]. These fatty alcohols make up 20–25% of the aliphatic chains in ciliary phospholipids, and we considered it possible that long-term adaptation to high temperatures might alter the composition of the ether linked groups. This proved to be the case; cilia from cells cultured at 35°C had significantly more 16 : 0 alcohol than 25°C controls (Table VC). When the sum of acyl-, amide- and ether-linked aliphatic chains is considered, the total percent unsaturation and total unsaturation index of ciliary membranes is not

TABLE V

CILIA FATTY ACIDS AND ETHER-LINKED ALCOHOLS FROM PHOSPHOLIPIDS AFTER 2 DAYS AT 25° AND 35°C

Cells were grown for two generations at 25°C and then either kept at 25°C or transferred to 35°C for 2 days at which time the cells were deciliated and extracted as described in Materials and Methods. The contributions of acyl (A), amide (B) and ether-linked (C) aliphatic chains are expressed as percent of total (D). The numbers represent means ± standard deviation. ($n = 2$).

Fatty acid	25°C	35°C
Acyl-linked (A)		
14:0	0.29 ± 0.06	0.20 ± 0.16
15:0	0.25 ± 0.01	0.21 ± 0.005
16:0	8.9 ± 1.1	5.7 ± 0.40
16:1	1.4 ± 0.65	0.75 ± 0.02
17.0	0.71 ± 0.69	0.04 ± 0.01
18:0	0.46 ± 0.08	0.44 ± 0.04
18:1	4.3 ± 0.20	4.4 ± 0.0
18:2	7.0 ± 0.20	5.5 ± 0.30
γ18:3	0.83 ± 0.17	0.34 ± 0.02
20:1	0.22 ± 0.13	1.0 ± 0.11
20:3	0.31 ± 0.02	1.1 ± 0.0
20:4	25.7 ± 2.2	25.9 ± 2.5
20:5	5.3 ± 0.40	8.0 ± 0.75
% unsat.	81.2 ± 3.1	87.7 ± 1.0
U.I.	152.2 ± 10.7	164.6 ± 14.3
Amide-linked (B)		
16:0	16.7 ± 0.45	7.2 ± 0.65
17:0	1.3 ± 0.0	0.99 ± 0.12
18:0	1.9 ± 0.15	5.4 ± 0.70
16:0/18:0	9.1 ± 1.0	1.4 ± 0.05
Ether-linked (C)		
16:0	18.7 ± 1.4	27.7 ± 4.4
18:1	6.2 ± 0.85	5.4 ± 1.7
Total (D)		
Total % unsat.	51.1 ± 2.8	52.3 ± 5.3
Total U.I.	158.5 ± 11.3	170.0 ± 16.0
% Acyl	55.4 ± 0.25	53.5 ± 4.2
% Amide	19.8 ± 0.30	13.6 ± 1.5
% Ether	24.8 ± 0.55	33.0 ± 2.7

different in 35°C cultures as compared with 25°C controls ($P > 0.1$ by Student's t test) (Table VD). The total 16 : 0 is also not different (the total percent 16 : 0 is 44.2 ± 2.0 and 40.6 ± 4.6 for 25 and 35°C, respectively).

Sterols of bodies and cilia. The kinds and amounts of sterol present in deciliated bodies were very similar for cells cultured for 2 days at 25 or

TABLE VI

CILIARY PHOSPHOLIPIDS AS A FUNCTION OF TIME AT 25 AND 35°C

Cells were grown for two generations at 25°C with $^{32}P_i$ (the inoculum was labeled for three generations with $^{32}P_i$) and then either kept at 25°C or transferred to 35°C. Cells returned to 25°C for 8 h were at 35°C for 8 h. At each time point the cells were deciliated and the ciliary phospholipids analyzed as described in Materials and Methods. The number in parentheses represents the time at each temperature. Only one analysis was done for the 25(8) and 35(8) samples but for the rest $n = 2$. The numbers represent means ± standard deviation. D and C are unidentified sphingolipids. Abbreviations are: GAEPL, 1-alkyl-2-acyl-*sn*-glycero-3-(2′-aminoethyl)phosphonate; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; GPC, 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholine; PI, phosphatidylinositol; CAEP, ceramide (2-aminoethyl)phosphonate; COPE, ceramide phosphorylethanolamine.

	GAEPL	PE	LPE	GPC	PI	CAEP	COPE	D	C
25°C (8)	45.3	8.2	3.7	3.4	1.0	16.5	2.5	8.0	2.4
35°C (8)	41.4	8.2	1.3	4.0	1.0	17.9	3.9	5.8	3.1
25°C (17)	46.6 ± 4.9	13.3 ± 5.2	2.4 ± 1.6	5.4 ± 1.8	1.3 ± 0.20	18.4 ± 0.70	1.9 ± 0.55	5.6 ± 1.0	1.7 ± 0.40
35°C (18)	49.4 ± 2.3	10.5 ± 1.6	0.65 ± 0.35	4.0 ± 0.80	1.1 ± 0.0	21.9 ± 0.10	2.8 ± 0.20	3.7 ± 0.45	1.2 ± 0.05
35 → 25°C (8)	51.5 ± 5.4	9.7 ± 1.9	2.7 ± 0.60	3.6 ± 1.3	1.3 ± 0.30	18.8 ± 1.10	2.1 ± 0.20	4.9 ± 0.60	1.3 ± 0.30

TABLE VII

SUMMARY OF CHANGES SEEN IN CULTURES SHIFTED FROM 25 TO 35°C FOR 8 h AND FOR 2 DAYS

The arrows represent the direction of the change in the percent of total fatty acids following transfer from 25 to 35°C. nc, represents no significant change. ($n = 2$)

	8 h		2 day	
	Body	Cilia	Body	Cilia
Total percent unsaturation	nc	↓7%	↑6%	nc
Total U.I.	nc	nc	↓3%	nc
Fatty acids				
16:0 (acyl)	nc	nc	↓41%	↓36%
18:0 (amide)	nc	↑200%	↑200%	↑280%
18:1 (acyl)	↑31%	nc	↑200%	NC
18:2 (acyl)	↓7%	nc	↓10%	↓21%
γ18:3 (acyl)	↓33%	↓200%	↓45%	↓240%
20:3 (acyl)	↑33%	nc	↑300%	↑350%
20:4 (acyl	nc	nc	↓20%	nc
20:5 (acyl)	nc	nc	nc	↑51%

35°C. The ratios of sterol to phospholipid were about 0.12 for both growth temperatures, and the ratoi of 7-dehydrocholesterol to 7-dehydrostigmasterol was 0.34. In the cilia of such cells, however, there were significant differences in sterol composition. Both the ratio of sterol to phospholipid and the ratio of 7-dehydrocholesterol to 7-dehydrostigmasterol were higher in 35°C cells (0.81 ± 0.14 vs. 0.48 ± 0.01 for 25°C controls and 0.71 ± 0.0 vs. 0.52 ± 0.0 in controls, respectively).

Phospholipid headgroups of cilia. The same 10 phospholipids were found in cilia of cells cultured at either 25 or 35°C, and most were present in the same relative proportions at both temperatures (Table VI). There were small but significant changes in three of the four sphingolipids at 35 vs. 25°C. The proportion of ceramide (2-aminoethyl)phosphonate and ceramide phosphorylethanolamine increased and that of sphingolipid D decreased. There was also a reproducible reduction in the amount of lysophosphatidylethanolamine at 35°C compared with 25°C controls. Each of these effects was reversed within 8 h when cultures were transferred back to 25°C from 35°C (Table VI). The phospholipid composition of deciliated bodies was not analyzed in these experiments.

Discussion

Our working hypothesis was that the lipid composition of the external membranes of *Paramecium* somehow influences thermosensory transduction. We used growth at high temperature to cause a perturbation of membrane lipid composition because changing the growth temperature has been shown to alter the membrane lipids of *Tetrahymena* [6,7,10,12,13,37–47] as well as many other organisms [1–5]. The involvement of membrane lipid in thermosensory transduction has also been suggested in other organisms [48]. We therefore tested our hypothesis by seeking changes in membrane lipid composition which correlated with changes in thermosensory behavior following a growth temperature shift.

The threshold for thermal avoidance of *Paramecium* is thermally adaptable. Cells grown at 25°C show a reversible loss of thermal avoidance of 42°C after a 4 h incubation at 35°C. The loss of thermal avoidance is sustained as long as the cells remain at 35°C. Membrane lipid composition of *Paramecium* also changes reversibly with growth temperature. In each of the five lipid components we studied (acyl-linked fatty acids, amide-linked fatty acids, long-chain hydrocarbons of glyceryl ethers, phospholipid headgroups, and sterols) significant changes occurred after a culture was shifted from 25 to 35°C. Three of these changes occurred within the same time scale as the change in thermal avoidance behavior, and were reversed after a culture was returned to its original growth temperature: (1) the ratio of 7-dehydrocholesterol to 7-dehydrostigmasterol in both ciliary and body membranes (compare Figs. 2 and 5), (2) the proportion of γ-linolenic acid (γ18 : 3) in membranes of body and cilia (Fig. 3) and (3) the relative proportions of ciliary lysophosphatidylethanolamine and sphingolipids (Table VI). Although the proportion of several individual fatty acids changed, the long-term effect of a temperature shift produced little change in the degree of total fatty acid unsaturation in phospholipids, whether measured as 'percentage unsaturation' or by the index of unsaturation. To summarize, specific lipids changed measurably in response to a temperature shift, but gross lipid unsaturation showed no sustained alteration (Table VII). The specific lipid changes occurred both in the ciliary membrane and in the membranes of deciliated bodies. The voltage-sensitive Ca^{2+} channels of the excitable membrane are exclusively localized in the ciliary membrane [49,50], but our preliminary evidence (Hennessey, Saimi and Kung, unpublished observations) suggests that thermoreceptors are located in the membranes of cell bodies.

Some of the changes in *Paramecium* ciliary lipids following a temperature shift were similar to the well-documented changes seen in the ciliate *Tetrahymena.* In both organisms, an increase in growth temperature produced a sustained increase in the proportion of ciliary γ18 : 3 and 18 : 0 and a decrease in the proportion of ciliary lysolipids [37,38]. Ohki et al. [40] analyzed the contributoin of each phospholipid class to microsomal membrane fluidity in *Tetrahymena,* and proposed that the level of γ18 : 3 in PnE was the primary determinant of long-term temperature-induced fluidity changes. In both *Tetrahymena* and *Paramecium,*

an unusually large amount of γ18 : 3 is found in the C-1 position of phospholipid, which normally carries a saturated fatty acid in most organisms [51]. Thus γ18 : 3 may be important in thermal acclimatization in both ciliates. *Paramecium* also showed a transient decrease in fatty acid unsaturation at high temperature, but this change did not persist as it does in *Tetrahymena.* In *Tetrahymena thermophilus* [52], one response to an increase in growth temperature is an increase in the proportion of ether-linked (saturated) acyl chains. Since in this species the major acyl-linked chain at the *sn*-C-1 position of glycerolipids is γ-linolenic (18 : 3), the result of increased glyceryl ether content is a decrease in unsaturation at higher temperatures.

In contrast with *Paramecium,* cilia of *Tetrahymena* showed a significant and sustained decrease in the proportion of unsaturated fatty acids when cells were incubated at high temperature. As a result of decreased fatty acid unsaturation, ciliary membranes of *Tetrahymena* grown at high temperatures became less fluid than membranes of cells cultured at low temperatures [42,45]. An example of the differences in fatty acid changes with temperature is the increase in the proportion of 16 : 0 in *Tetrahymena* microsomal membranes that occurs during growth at 39.5°C concomitant with a decrease in the activity of a palmitoyl-CoA desaturase [12,13]. In *Paramecium* the proportion of acyl and amide-linked 16 : 0 are reproducibly decreased in cilia after 2 days at 35°C but because ether-linked 16 : 0 increases, the total 16 : 0 in cilia is not different.

Recently, Thompson and coworkers have characterized the lipids of microsomal and ciliary membranes of *Tetrahymena* after a shift in growth temperature from 39 to 15°C ('low temperature acclimation') [42,45]. Both microsomal and ciliary membranes changed in fatty acid composition after the temperature shift, acquiring higher proportions of 18 : 2 [6,11] and 18 : 3 at the lower temperature, and thereby increasing the index of unsaturation. The change occurred sooner in microsomal membranes than in ciliary membranes, but the final extent of the change was greater in the ciliary membranes. The fluidity, assayed by fluorescence depolarization of the extrinsic probe DPH was greater in ciliary membranes from cells shifted to 15°C.

In addition to these net changes in fatty acid composition, Ramesha et al. also showed striking redistributions of fatty acids among the several phospholipid species of ciliary membranes after a shift down in growth temperature [44]. Dickens and Thompson [43] and Maruyama et al. [47] found such redistributions in acyl chains to occur in microsomes soon after a shift down in temperature. It seems entirely likely that, in addition to the net changes in fatty acid composition which we see in ciliary membranes of *Paramecium,* there is also a redistribution of fatty acids among individual phospholipid species, for which we have not yet looked.

One possible reason for the difference between the temperature-induced lipid changes in *Tetrahymena* and *Paramecium* is the differences in their lipid metabolism. *Paramecium,* unlike *Tetrahymena* [15], cannot make fatty acids de novo from acetate but synthesizes most of its fatty acids from dietary oleate [53]. *Tetrahymena* also synthesizes the sterol-like molecule tetrahymenol [54] whereas *Paramecium* requires exogenous sterol for growth [36]. The only metabolic conversion of sterols in *Paramecium* is a dehydrogenation at position 7 of a sterol such as cholesterol or stigmasterol and esterification of fatty acids to the hydroxyl group of certain sterols [36]. *Paramecium* is therefore an auxotroph for unsaturated fatty acids and for sterol whereas *Tetrahymena* is not. Another possible explanation for the differences between the temperature-induced lipid changes of *Tetrahymena* and *Paramecium* is that in *Paramecium* we studied cells incubated at 25 and 35°C, while in *Tetrahymena* studies cells were grown at 15 and 39.5°C. We chose to examine *Paramecium* at 25 and 35°C because sustained and reversible behavioral changes could be seen at these temperatures.

The lipid modifications induced by changes in growth temperature may affect thermosensory transduction indirectly by altering the function of intrinsic membrane protein thermoreceptors. Alternatively, the membrane lipid may itself act as the primary thermosensor. Intrinsic membrane proteins function in a lipid environment, and there is good evidence that the physical state of associated lipids can influence the activity of membrane proteins [19–24]. It is possible that in *Paramecium* the primary thermoreceptor is a lipid or lipids,

associated with a membrane protein (perhaps an ion channel), which undergoes a solid-to-fluid transition at the threshold temperature of thermal avoidance. According to this model, when a cell enters a warmer region, a phase transition in the lipid influences the function of the associated protein, and the result is a thermosensory transduction producing a thermal avoidance response. A change in this transition temperature (resulting, for example, from a change in the amount of $\gamma 18:3$ in the phospholipid) would shift the protein's thermosensory range.

It is also possible that some protein that is essential to thermoreception is altered at high growth temperature. In fact, Adoutte et al. [55] found several differences between the ciliary membrane proteins of *Paramecium* cultured at 25 and 35°C, and there are many known examples of reversible, covalent alterations of proteins, which affect function. The results we describe here are consistent with the hypothesis that lipid phase transitions play a role in thermosensation, but they do not provide definitive proof of that hypothesis.

Note added in proof: (Received January 7th, 1983)

Sommerville and McTavish (Biochim. Biophys. Acta 698, 158–166, (1982)) showed that when paramecia grown at 24°C were shifted to 32°C, the rate of protein synthesis increased immediately, and a large number of new mRNA species appeared.

Acknowledgments

This research was supported by grants from the National Science Foundation (BNS 76-11490 and BNS 81-00832), the Graduate School of the University of Wisconsin and a Dreyfus Foundation Teacher-Scholar Award to D.L.N. D.L.N. is recipient of a Research Career Development Award (NIH 00085) and a Steenbock Career Advancement Award. We thank Michael Forte and Bruce Whitaker for reading the manuscript.

References

1 Hazel, J.R. and Prosser, C.R. (1974) Physiol. Rev. 54, 620–677
2 Sinensky, M. (1974) Proc. Natl. Acad. Sci. USA 71, 522–525
3 Cossins, A.R. (1977) Biochim. Biophys. Acta 470, 395–411
4 Wodtke, E. (1981) Biochim. Biophys. Acta 640, 698–709
5 Anderson, R.L., Minton, K.W., Li, G.C. and Hahn, G.M. (1981) Biochim. Biophys. Acta 641, 334–348
6 Thompson, G.A. and Nozawa, Y. (1977) Biochim. Biophys. Acta 472, 55–92
7 Ferguson, K.A., Conner, R.L. and Mallory, F.B. (1971) Arch. Biochim. Biophys. 144, 448–450
8 Ferguson, K.A., Davis, F.M., Conner, R.L., Landrey, J.R. and Mallory, F.B. (1975) J. Biol. Chem. 250, 6998–7005
9 Nozawa, Y., Fukushima, H. and Iida, H. (1975) Biochim. Biophys. Acta 406, 248–263
10 Kitajima, Y. and Thompson, G.A. (1977) Biochim. Biophys. Acta 468, 73–80
11 Nandini-Kishore, S.G., Kitajima, Y. and Thompson, G.A. (1977) Biochim. Biophys. Acta 471, 157–161
12 Martin, C.E., Hiramitsu, K., Kitajima, Y., Nozawa, Y., Skriver, L. and Thompson, G.A. (1976) Biochemistry 15, 5218–5227
13 Kasai, R., Kitajima, Y., Martin, C.E., Nozawa, Y., Skriver, L. and Thompson, G.A. (1976) Biochemistry 15, 5228–5233
14 Mavis, R.D. and Vagelos, P.R. (1972) J. Biol. Chem. 247, 652–659
15 Rintoul, D.A., Sklar, L.A. and Simoni, R.O. (1978) J. Biol. Chem. 253, 7447–7452
16 Schroeder, F. (1978) Nature 276, 528–530
17 Stubbs, C.O., Tsang, W.M., Belin, J., Smith, A.D. and Johnson, S.M. (1980) Biochemistry 19, 2756–2762
18 Herring, F.G., Tatischeff, I. and Weeks, G. (1980) Biochim. Biophys. Acta 602, 1–9
19 Lenaz, G., Curatola, G. and Masotti, L. (1975) J. Bioenerg. 7, 223–299
20 Kimelberg, H.K. (1977) in Cell Surface Reviews (Poste, G. and Nicolson, G.L., eds.), Vol. 3, pp. 205–293, North-Holland
21 Gazzotti, P. and Peterson, S.W. (1977) J. Bioenerg. Biomemb. 9, 373–386
22 Sandermann, H. (1978) Biochim. Biophys. Acta 515, 209–237
23 Shinitzky, M. and Henkart, P. (1979) Int. Rev. Cytol. 60, 121–147
24 Quinn, P.J. and Chapman, F. (1980) CRC Crit. Rev. Biochem. 8, 1–177
25 Tanabe, H., Kurihara, K. and Kabatake, Y. (1979) Biochim. Biophys. Acta 553, 396–403
26 Hennessey, T.M. and Nelson, D.L. (1979) J. Gen. Microbiol. 112, 337–347
27 Forte, M., Satow, Y., Nelson, D.L. and Kung, C. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 7195–7199
28 Soldo, A.T. and Van Wagtendonk, W.J. (1969) J. Protozool. 16, 500–506
29 Hennessey, T.M., Andrews, D. and Nelson, D.L. (1983) J. Lipid Res., in the press
30 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917
31 Andrews, D. and Nelson, D.L. (1979) Biochim. Biophys. Acta 550, 174–187
32 Kates, M. (1972) Techniques of lipidology. Isolation, Anal-

ysis and Identification of Lipids (Work, T.S. and Work, E., eds.), North-Holland, New York
33 Rhoads, D.E. and Kaneshiro, E.S. (1979) J. Protozool. 28, 329–338
34 Rhoads, D.E., Meyer, K.B. and Kaneshiro, E.S. (1981) Biochem. Biophys. Res. Commun. 98, 858–865
35 Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758
36 Conner, R.L., Landrey, J.R., Kaneshiro, E.S. and Van Wagtendonk, W.J. (1971) Biochim. Biophys. Acta 239, 312–319
37 Nozawa, Y., Iida, H., Fukushima, H., Ohki, K. and Ohnishi, S. (1974) Biochim. Biophys. Acta 367, 134–147
38 Fukushima, H., Martin, C.E., Iida, H., Kitajima, Y., Thompson, G.A. and Nozawa, Y. (1976a) Biochim. Biophys. Acta 431, 165–179
39 Martin, C.E. and Thompson, G.A. (1978) Biochemistry 17, 3581–3586
40 Ohki, K., Kasai, R. and Nozawa, Y. (1979) Biochim. Biophys. Acta 558, 273–281
41 Watanabe, T., Fukushima, H. and Nozawa, Y. (1980) Biochim. Biophys. Acta 620, 133–141
42 Dickens, B.F. and Thompson, G.A., Jr. (1981) Biochim. Biophys. Acta 644, 211–218
43 Dickens, B.F. and Thompson, G.A., Jr. (1982) Biochemistry 21, 3604–3611
44 Ramesha, C.S., Dickens, B.F. and Thompson, G.A., Jr. (1982) Biochemistry 21, 3618–3629
45 Ramesha, C.S. and Thompson, G.A., Jr. (1982) Biochemistry 21, 3612–3617
46 Watanabe, T., Fukushima, H., Kasai, R., and Nozawa, Y. (1981) Biochim. Biophys. Acta 665, 66–73
47 Maruyama, H., Banno, Y., Watanabe, T. and Nozawa, Y. (1982) Biochim. Biophys. Acta 711, 229–244
48 Poff, K.L. and Skokut, M. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 2007–2010
49 Dunlop, K. (1977) J. Physiol. 271, 119–134
50 Ogura, A. and Takahashi, K. (1976) Nature 264, 170–172
51 Kaneshiro, E.S. (1980) J. Lipid Res. 21, 559–570
52 Lund-Katz, S. and Conner, R.L. (1981) Biochem. Biophys. Res. Commun. 101, 837–845
53 Rhoads, D.E. and Kaneshiro, E.S. (1980) J. Cell Biol. 87, 326a
54 Beedle, A.S., Munday, K.A. and Wilton, D.C. Biochem. J. 142, 57–64
55 Adoutte, A., Ling, K-Y., Chang, S., Huang, F. and Kung, C. (1983) J. Cell Biol., in the press