Changes in the Lipid Composition and Physical Properties of *Tetrahymena* Ciliary Membranes following Low-Temperature Acclimation†

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ABSTRACT: This report describes the composition and physical properties of ciliary membranes isolated from *Tetrahymena pyriformis* grown at 39 and 15 °C. These cell-surface membranes differed from the more commonly studied microsomal membranes in having much higher levels of the sterol-like triterpenoid tetrahymanol, smaller amounts of phosphatidylethanolamine and phosphatidylcholine, and appreciable quantities of two sphingolipids. The changes in the ciliary phospholipid distribution and fatty acid pattern found in 15 °C grown cells were generally similar to but considerably more pronounced than those in microsomal membranes. Yet the physical properties of ciliary lipids and native membranes, as monitored by fluorescence depolarization of the probe diphenylhexatriene, showed fewer distinctive changes at low temperature than did equivalent preparations from microsomes. Ciliary preparations from both 39 and 15 °C grown cells lacked the recognizable break points always observed in polarization vs. temperature plots of microsomal lipids and membranes. In sharp contrast to the almost immediately detectable response of microsomal lipid composition to chilling [Dickens, B. F., & Thompson, G. A., Jr. (1981) *Biochim. Biophys. Acta 644*, 211], the characteristic changes in ciliary phospholipid fatty acids of cells chilled from 39 to 15 °C began only after 8 h at the low temperature. This finding, coupled with data comparing the specific radioactivity of [^{14}C]palmitate-labeled microsomal and ciliary lipids, indicated a very slow exchange of phospholipids between microsomes and cilia. The retarded lipid dissemination may be partly responsible for the 15-h period needed for the resumption of cell growth at 15 °C.

Tetrahymena pyriformis has been extensively utilized as a model system for studying membrane lipid dynamics (Nozawa & Thompson, 1979). In recent work, including the preceding paper (Dickens & Thompson, 1982), our laboratory has characterized the detailed lipid changes induced in *Tetrahymena* microsomal membranes by a change in environmental temperature. These changes brought about by the microsomal enzymes permit a significant fluidization of the microsomal membrane within minutes following exposure to low temperature.

In contrast, the transfer of these rapidly modified microsomal lipids to other cellular membranes of chilled *Tetrahymena* is very slow (Nozawa & Thompson, 1971b) and might constitute the rate-limiting step in the organism's adaptation process. In this paper, we describe changes occurring in the lipid composition and physical properties of *Tetrahymena* surface membranes as the indirect result of microsomal enzyme activity. The findings strengthen the concept of a sluggish intracellular lipid movement and confirm a definite molecular selectivity of lipid dissemination.

Materials and Methods

Materials

1,6-Diphenyl-1,3,5-hexatriene (DPH) was purchased from Aldrich Chemical Co. (Milwaukee, WI) and was used without further purification. β-Amyrin was purchased from Sigma Chemical Co. (St. Louis, MO). Its purity was confirmed by gas–liquid chromatography.

[1-^{14}C]Palmitic acid (specific activity 56.3 Ci/mol) was purchased from New England Nuclear (Boston, MA). Its radiopurity was confirmed by thin-layer chromatography (TLC).

Methods

(*i*) *Culture Conditions. Tetrahymena pyriformis*, strain NT-1, was grown in 2% proteose peptone medium as previously described by Fukushima et al. (1976). The cells were grown isothermally at either 39 or 15 °C and were harvested in their mid-logarithmic growth phase [(2.0–2.8) × 10^5 cells/mL].

(*ii*) *Deciliation.* Cells were deciliated by the following modification of a procedure used by Adoutte et al. (1980) for deciliating *Paramecium*. Cells were harvested by centrifugation at 300*g* for 5 min and then washed once with Tris–EDTA–sucrose buffer (20 mM Tris, 10 mM EDTA, and 125 mM sucrose, pH 7.2). The washed cells were suspended in acetate–EDTA–sucrose buffer (80 mM sodium acetate, 10 mM EDTA, and 125 mM sucrose, pH 6.8) to a final density of (4.5–5.5) × 10^8 cells/mL. After 5–6 min, a solution of 0.2 M $CaCl_2$ was added to a final Ca^{2+} concentration of 10 mM, and the cell suspension was gently swirled from time to time. Ten minutes after the addition of $CaCl_2$, the suspension was gently passed 2–3 times through a no. 18 gauge hypodermic needle into a 20-mL syringe. This procedure detached 70–80% of the cilia with no blistering or lysis of the cells. The suspension was diluted with 3 volumes of Tris–EDTA–sucrose buffer (pH 7.2) and centrifuged at 480*g* for 5 min. The supernatant was recentrifuged under the same conditions to sediment any remaining cell bodies. The final supernatant was then centrifuged at 18000*g* for 15 min to pellet out cilia. The ciliary pellet was suspended either in saline or in Tris–EDTA buffer (1.0 mM Tris and 0.2 mM EDTA, pH 8.4). All procedures starting from harvesting of cells to final suspension of cilia were carried out at 2–4 °C. The purity of cilia was confirmed by phase-contrast and electron microscopy.

(*iii*) *Isolation of Ciliary Membrane.* Ciliary membrane was removed by vigorously vortexing the ciliary pellet in Tris–EDTA buffer (1.0 mM Tris and 0.2 mM EDTA, pH 8.4) for 2–3 min, and the detached membrane vesicles were recovered by centrifuging the vortexed ciliary suspension on a discontinuous sucrose density gradient as described by Adoutte et al. (1980).

(*iv*) *Lipid Analysis.* Lipids from cilia were extracted by the

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Table I: Phospholipid Composition of Cilia from 39 and 15 °C Grown *Tetrahymena*[a]

phospholipid	39 °C grown	15 °C grown
2-(aminoethyl)phosphonolipid	40.0 ± 2.2	42.8 ± 1.2
phosphatidylethanolamine	18.4 ± 2.0	9.9 ± 1.2
phosphatidylcholine	15.7 ± 3.7	3.1 ± 0.6
ceramide (aminoethyl)phosphonate	3.0 ± 1.7	37.4 ± 2.2
ceramide aminoethyl phosphate	22.8 ± 1.5	6.8 ± 1.3
phospholipid:tetrahymanol molar ratio	2.56 ± 0.36	4.23 ± 0.23

[a] Values are expressed as mole percent and are mean ± SD from four or more analyses.

method of Bligh & Dyer (1959). Separation of neutral lipids and phospholipids and analysis of their fatty acid composition by gas–liquid chromatography (GLC) have been described earlier (Fukushima et al., 1976). Lipid phosphorus was estimated by the method of Bartlett (1959) as modified by Marinetti (1962).

Tetrahymanol was quantified by GLC on a 3% OV-1 column with β-amyrin as the internal standard (Thompson et al., 1971).

(*v*) *Fluorescence Measurements.* Fluorescence polarization measurements of 1,6-diphenylhexatriene (DPH) in multibilayer vesicles of ciliary total lipids and phospholipids and in ciliary membrane vesicles were done essentially as described earlier (Dickens & Thompson, 1980).

(*vi*) *[1-^{14}C]Palmitate Incorporation.* Incorporation of [1-^{14}C]palmitic acid by *Tetrahymena* was carried out as follows: 2.3 × 10^6 dpm of [1-^{14}C]palmitate (specific activity 56.3 Ci/mol) in 100 μL of ethanol was added to a 200-mL logarithmic phase [(2–3) × 10^5 cells/mL] culture of *Tetrahymena pyriformis* at 39 °C. Ten minutes after the addition of the palmitic acid, the flasks were chilled down to 15 °C as described earlier (Dickens & Thompson, 1981) and then incubated at 15 °C for specified periods of time, after which cells were harvested and their ciliary and microsomal lipids isolated (Nozawa & Thompson, 1971a). Different phospholipid classes were then separated by TLC, and their lipid phosphorus content and radioactivities were measured as described elsewhere (Fukushima et al., 1976; Nozawa & Thompson, 1971b).

Results

Distribution of Phospholipid Classes. Earlier reports (Nozawa & Thompson, 1971a) showed sizable differences between *Tetrahymena* microsomes and cilia with respect to their phospholipid composition. We have refined the earlier data by starting with a more highly purified preparation of cilia and using improved chromatographic separation techniques. The proportions of the different phospholipid classes in cilia (Table I) were very different from those in microsomes (Dickens & Thompson, 1982), and there were also major differences between cilia isolated from 39 and 15 °C grown cells. The amounts of ciliary phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were greatly reduced in cells grown at lower temperature, while the major constituent, 2-(aminoethyl)phosphonolipid (AEPL), showed little change. The most striking difference between 39 and 15 °C cilia was in the relative proportions of two ceramide-containing phospholipids. Based on their comigration with authentic lipid standards on thin-layer chromatograms and on studies of their hydrolytic products, we have tentatively identified these lipids as ceramide (aminoethyl)phosphonate (CAEP) and ceramide aminoethyl phosphate (CPE).

The phospholipid:tetrahymanol molar ratio in ciliary lipids from 39 °C grown cells was 2.56 ± 0.36, considerably lower than the ratio of 4.23 ± 0.23 found in cilia from 15 °C grown cells. Both values were very much lower than the growth temperature insensitive ratio of 14.23 found in microsomes (Dickens & Thompson, 1982).

Table II: Overall Fatty Acid Composition of Ciliary Phospholipids of *Tetrahymena* Grown at Different Temperatures[a]

fatty acid	39 °C grown	15 °C grown
14:0	14.9 ± 3.3	7.3 ± 0.1
iso 15:0	4.9 ± 0.5	0.9 ± 0.2
15:0	1.8 ± 0.3	tr[b]
16:0	16.3 ± 0.5	14.9 ± 1.8
16:1	11.5 ± 1.5	6.5 ± 0.5
16:2 + 17:0 + 17:1	3.8 ± 0.4	2.9 ± 0.5
18:0	1.9 ± 0.4	2.5 ± 0.2
18:1	5.0 ± 0.9	4.8 ± 0.6
18:2($\Delta^{6,11}$)	2.5 ± 0.3	17.3 ± 0.5
18:2($\Delta^{9,12}$)	8.6 ± 1.0	5.5 ± 0.7
18:3	27.9 ± 2.6	37.0 ± 5.5

[a] Values are expressed as mole percent and are mean ± SD of three or more experiments. Values are not corrected for the presence of ether side chains. [b] tr represents values <0.5%.

The ciliary lipids isolated from 15 °C grown cells were considerably more unsaturated than those isolated from 39 °C grown cells (Table II). The higher unsaturation of the former was mainly due to an elevated proportion of γ-linolenic acid [18:3($\Delta^{6,9,12}$)] and cilienic acid [18:2($\Delta^{6,11}$)], with the latter fatty acid being enriched by about 8-fold in ciliary lipids of 15 °C grown cells.

To assess how temperature affects the fatty acid composition of individual phosphoglyceride classes and to compare these differences with those observed in microsomal membranes from the same cells, we purified the phospholipid classes by TLC and analyzed the fatty acid composition of the three major phosphoglycerides. Ciliary AEPL from both 39 and 15 °C grown cells contained γ-linolenic acid as the major fatty acid (Table III). Ciliary AEPL from 15 °C grown cells had comparatively high amounts of cilienic acid while that from 39 °C grown cells had high proportions of palmitic and palmitoleic acids. AEPL from both 39 and 15 °C cilia also contained extremely high amounts (42–46%) of alkyl ether side chains, mainly of the 16:0 species.

Ciliary PE from both 39 and 15 °C grown cells contained palmitic acid as the major fatty acid. Ciliary PE from 15 °C grown cells had significantly higher amounts of oleic acid while that from 39 °C cells was enriched in 15:0. Interestingly, cilienic acid was absent in ciliary PE of both 39 and 15 °C grown cells.

Analysis of the ciliary PC fatty acid composition revealed more striking differences between 15 and 39 °C grown cells than were seen in AEPL or PE. Thus, ciliary PC from 39 °C grown cells had palmitic acid as the major fatty acid. It also contained a relatively high level of 15:0 but no cilienic acid, while that from 15 °C grown cells had myristic acid as its major fatty acid and contained a significantly higher amount of cilienic acid than did PC from 39 °C grown cilia.

Unlike the glycerophospholipids, which contained only nonhydroxy fatty acids, the two sphingolipids CAEP and CPE had a sizable content of hydroxy fatty acids. Preliminary studies have detected noteworthy differences, both qualitative and quantitative, in the distribution of the nonhydroxy and the hydroxy fatty acids, with the latter type showing the greatest variation. Hydroxytetradecanoic acid (14h:0) and hydroxyhexadecanoic acid (16h:0) were present in almost equal proportion in the ciliary CPE of 39 °C grown cells and together constituted approximately 70% of this lipid's total fatty acids. However, ciliary CPE from 15 °C grown cells contained a

Table III: Fatty Acid Composition of Individual Phospholipid Classes Isolated from Cilia of 39 and 15 °C Grown *Tetrahymena*[a]

	2-(aminoethyl)phosphonolipid		phosphatidylethanolamine		phosphatidylcholine	
fatty acid	39 °C	15 °C	39 °C	15 °C	39 °C	15 °C
12:0	tr[b]	tr	tr	tr	1.7 ± 0.3	0.9 ± 0.1
14:0	3.2 ± 0.2	2.8 ± 0.7	25.7 ± 0.5	20.5 ± 3.4	18.9 ± 0.2	26.8 ± 5.4
iso 15:0	tr	tr	8.9 ± 0.5	2.6 ± 0.9	6.4 ± 0.1	2.8 ± 0.3
15:0	0.9 ± 0.1	tr	4.2 ± 0.5	tr	3.8 ± 0.1	tr
16:0	4.1 ± 0.2	2.3 ± 0.1	24.0 ± 2.4	23.9 ± 4.5	19.4 ± 0.1	15.8 ± 0.7
16:1	4.7 ± 0.1	2.5 ± 0.2	15.5 ± 2.0	18.2 ± 1.1	10.1 ± 0.1	9.0 ± 0.1
16:2 + 17:0 + 17:1	1.8 ± 0.4	tr	4.6 ± 1.1	4.5 ± 2.0	3.6 ± 0.1	2.8 ± 0.7
18:0	tr	tr	2.1 ± 0.3	1.2 ± 0.2	1.3 ± 0.1	1.2 ± 0.1
18:1	2.5 ± 0.7	2.4 ± 0.1	4.9 ± 0.9	12.9 ± 0.4	5.2 ± 0.1	4.4 ± 1.4
18:2($\Delta^{6,11}$)	4.4 ± 0.3	16.0 ± 1.2				3.2 ± 0.3
18:2($\Delta^{9,12}$)	4.4 ± 0.3	3.5 ± 0.8	7.0 ± 0.2	9.8 ± 2.2	5.1 ± 1.1	3.9 ± 0.5
18:3	23.9 ± 0.3	23.5 ± 1.5	2.1 ± 0.7	5.5 ± 2.5	6.5 ± 0.1	9.7 ± 1.9
e16:0 + e16:1[c]	48.8 ± 0.3	45.2 ± 1.2			17.8 ± 0.1	19.4 ± 0.5

[a] Values are expressed as mole percent and are mean ± SD of three or more experiments. [b] tr represents values <0.5%. [c] e denotes alkyl ether side chain.

much lower level of hydroxy fatty acids, with 14h:0 and 16h:0 accounting for 10% and 30%, respectively, of the total fatty acid complement.

The other major ciliary sphingolipid, CAEP, of 39 °C grown cells resembled CPE in having a significant amount (20%) of 14h:0, but another 55% of the total fatty acids was present as hydroxyoctadecanoic acid (18h:0), a component not detected in CPE. Here too, the composition was altered in 15 °C grown cilia so that the complement of hydroxy fatty acids, which in this case accounted for approximately 35% of the total fatty acids, was made up of roughly equal parts of 16h:0, 17h:0, and 18h:0. Because the present report is primarily a comparison of lipids occurring in ciliary as well as microsomal membranes, a detailed characterization of the ceramide lipids will be presented elsewhere.

Physical Properties of Isolated Ciliary Lipids and Intact Ciliary Membrane. Very characteristic temperature-induced differences in the physical properties of 39 and 15 °C grown *Tetrahymena* microsomes and microsomal lipids have been found by measuring the fluorescence polarization of a hydrophobic probe, diphenylhexatriene (DPH) (Dickens & Thompson, 1980). Similar measurements have now been made by using ciliary preparations.

Panels A and B, respectively, of Figure 1 show typical polarization vs. temperature (P vs. T) plots of DPH in ciliary total lipids and phospholipids isolated from 39 and 15 °C grown cells. These plots resemble the same type of plots obtained with microsomal lipids (Dickens et al., 1980) in one respect. Namely, at any given temperature, the polarization values for ciliary total lipids and phospholipids from 15 °C cells were lower than those for equivalent preparations obtained from 39 °C grown cells, indicating the greater fluidity of the lipids from 15 °C cells. This was expected because of the higher content of unsaturated fatty acids in the 15 °C ciliary lipids. However, whereas the slopes of P vs. T curves obtained by using microsomal phospholipids and total lipids were similar (compare inset of Figure 1A with that of Figure 1B), this was not the case with ciliary preparations. The ciliary total lipids yielded DPH polarization values equal to or lower than those in phospholipid samples at low temperature but considerably higher than those in phospholipids at high temperature (Figure 1A,B). This difference in slope between the ciliary total lipid and phospholipid curves reflects the fluidity-stabilizing effect of tetrahymanol, which comprises a substantial proportion of the ciliary total lipids but is not a major lipid of microsomes.

It is also interesting to note that in contrast to the two distinct "break points", or abrupt changes in slope, present in the P vs. T curves of microsomal lipids, P vs. T curves from either ciliary total lipids or ciliary phospholipids showed no discrete break points. Many P vs. T curves displayed slight indications of nonlinear slope change, but our methods for verifying slope change (Dickens & Thompson, 1980) were not sufficiently sensitive to identify specific break-point temperatures.

Figure 1C shows the polarization vs. temperature curves of DPH in intact ciliary membranes isolated from 39 and 15 °C grown cells. Here also the ciliary membranes from 15 °C grown cells were more fluid than those isolated from 39 °C grown cells. But when these polarization values were compared with those in Figure 1A,B, it could be seen that intact ciliary membranes from 39 and 15 °C cells were less fluid than were the total lipids extracted from them. However, the slopes of the P vs. T curves for native membranes were similar to those of total lipids.

Changes in Ciliary Lipid Composition with Time after Chilling. The distribution of different phospholipid polar head groups remained unchanged during the 12-h period following a temperature shift. Analysis of cilia harvested at 2, 8, and 12 h following chilling (data not shown) detected no significant changes in the levels of AEPL, PE, PC, and the two types of sphingolipids, and the values were comparable with those found in cells fully acclimated to 39 °C (see Table I). This lack of rapid change in polar head group composition was perhaps to be expected, since even chilled microsomal membranes are slow to change in this respect (Dickens & Thompson, 1981).

On the other hand, the fatty acid composition of *Tetrahymena* microsomal lipids underwent rapid changes following a shift of culture temperature from 39 to 15 °C (Dickens & Thompson, 1981). A large increase in fatty acid unsaturation was observed during the first 4 h with significant changes being noted as early as 1 h after chilling. It is therefore noteworthy that the fatty acid composition of ciliary phospholipids measured at various times following the shift from 39 to 15 °C (Table IV) did not reflect these rapid changes that were taking place in microsomes. No really consistent alterations were apparent except for a rise in palmitoleic acid (16:1), which attained a maximum content approximately 8 h following the temperature shift and then declined. The tendency of 16:1 to become temporarily elevated in chilled *Tetrahymena* has been noted by several investigators [e.g., see Nozawa & Kasai (1978) and Martin & Thompson (1978)].

Fatty acid analyses of purified ciliary AEPL, PE, and PC (data not shown) at 4, 8, and 12 h following the shift from 39 to 15 °C showed no consistent trend of change toward the

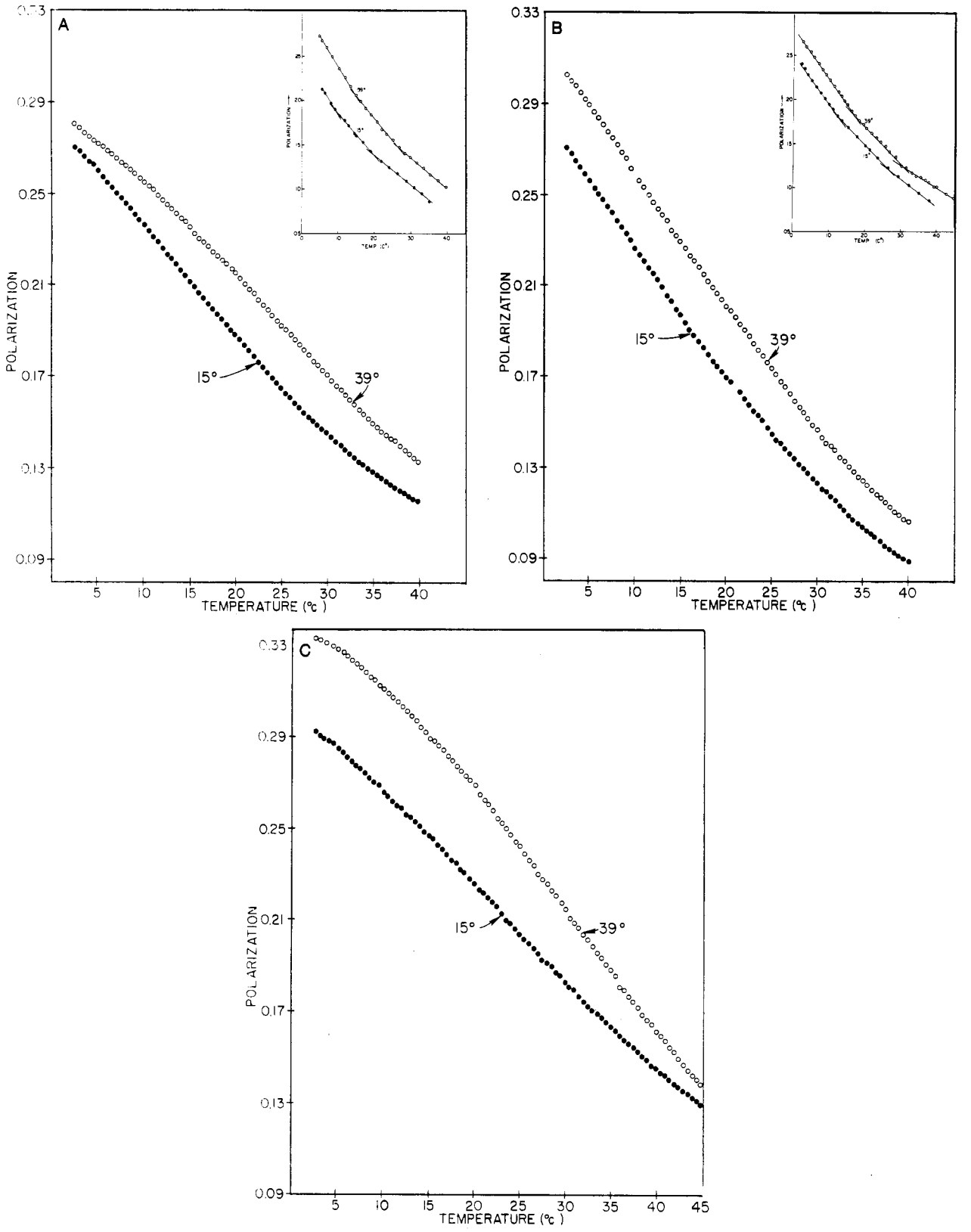

FIGURE 1: Influence of temperature on diphenylhexatriene polarization in multibilayer vesicles of ciliary total lipids (A) and phospholipids (B) and in membrane vesicles (C). Insets: Polarization vs. temperature plots of corresponding microsomal preparations [from Dickens et al. (1980)].

patterns found in those lipids of 15 °C grown cilia.

Redistribution of Radioactive Phospholipids during the Acclimation Process. To gain further insight into the sluggish rate of lipid transport from microsomes to cilia, we followed the redistribution of [^{14}C]palmitate prelabeled phospholipid from microsomes to cilia throughout a period of several hours after the temperature shift. During the 5–10-min period following radioisotope addition at 39 °C, there was a measurable incorporation [(2–8) × 10^4 cpm/μmol of phosphorus] of [^{14}C]palmitate into each of the three major ciliary phospholipids, catalyzed by fatty acyl transferases localized in outer parts of the cell. But, as shown earlier (Nozawa & Thompson,

Table IV: Effects of Chilling on the Fatty Acid Composition of Ciliary Phospholipids[a]

fatty acid	39 °C grown	39 → 15 °C (4 h)	39 → 15 °C (8 h)	39 → 15 °C (12 h)	15 °C grown
14:0	15.0 ± 3.3	16.1 ± 2.6	13.1 ± 0.8	11.0 ± 0.7	7.3 ± 0.1
iso 15:0	4.9 ± 0.5	5.1 ± 0.4	3.9 ± 0.4	3.3 ± 0.4	0.9 ± 0.2
15:0	1.8 ± 0.3	2.6 ± 0.3	1.8 ± 0.2	2.0 ± 0.2	tr
16:0	16.4 ± 0.5	13.4 ± 1.0	10.4 ± 1.4	9.5 ± 0.6	14.8 ± 1.8
16:1	11.5 ± 1.5	16.8 ± 1.2	18.5 ± 1.0	15.2 ± 0.6	6.6 ± 0.6
16:2 + 17:0 + 17:1	3.8 ± 1.3	5.4 ± 0.8	6.1 ± 1.2	6.1 ± 0.7	2.9 ± 0.5
18:0	1.9 ± 0.4	1.3 ± 0.3	1.2 ± 0.3	1.1 ± 0.2	2.5 ± 0.2
18:1	5.0 ± 0.9	4.0 ± 0.6	6.9 ± 0.4	6.8 ± 0.5	4.7 ± 0.6
18:2($\Delta^{6,11}$)	2.5 ± 0.3	1.3 ± 0.5	2.9 ± 1.2	4.0 ± 0.3	17.2 ± 0.5
18:2($\Delta^{9,12}$)	8.7 ± 1.0	8.6 ± 0.9	9.7 ± 0.3	8.9 ± 0.9	5.4 ± 0.7
18:3	28.0 ± 2.6	23.1 ± 1.7	24.8 ± 2.1	31.7 ± 1.0	36.7 ± 5.4

[a] Values are expressed as mole percent and are mean ± SD of three or more analyses. Values are not corrected for the amounts of ether side chains.

1971b), microsomal phospholipids attained a 10-fold higher specific radioactivity during the period of [^{14}C]palmitate uptake. After being chilled, the specific radioactivities of the individual prelabeled ciliary phosphoglycerides all remained relatively constant at their lower values for at least 12 h at 15 °C. These data complement the studies of fatty acid compositional change (Table IV) in indicating that lipids are disseminated from microsomes into cilia at a very slow rate.

Discussion

Lipid Differences between Microsomes and Cilia. Scattered evidence from earlier reports (Nozawa & Thompson, 1971b; Martin & Thompson, 1978) suggested that other functionally different *Tetrahymena* membranes were much slower in their response to low temperature than were microsomes. The membrane most remote from the microsomes in a physical and a metabolic sense is that enclosing the cilia. As such, the ciliary membrane should be well suited for examining the dynamics and selectivity of intracellular lipid movement in times of stress.

Although ciliary lipids were in some respects similar to the lipids of microsomes, they also differed in many qualitative and quantitative ways. The three major phospholipid classes of microsomal phospholipids [see Table I of Dickens & Thompson (1982)] were also represented in the ciliary membrane (Table I), but in strikingly different proportions, and the ciliary membrane contained sphingolipids which were found only in trace amounts in microsomes. The phospholipid:tetrahymanol ratio was 4–7 times lower in cilia than in microsomes.

Temperature-Induced Changes in Ciliary Lipids. Ciliary membranes exhibited large differences in lipid composition when cultured at the two extreme temperatures (Tables I–III). The trends were generally similar to those found in the microsomes (Fukushima et al., 1976; Dickens & Thompson, 1982) and, in some cases, even more pronounced. Two sphingolipids, which were barely detectable in microsomes, were prevalent in cilia and differed markedly in their levels, depending upon the growth temperature.

The fatty acid patterns of the individual ciliary phosphoglycerides were distinct from those of their microsomal counterparts. Ciliary fatty acid differences between high and low temperature grown cells followed the same trends observed in microsomes, even though they were in many instances superimposed on dissimilar basal levels.

Thus, phospholipids were disseminated to the ciliary membrane from the site of their synthesis in microsomes in a selective fashion with respect to phospholipid classes and with respect to fatty acid composition within each class. The fact that the compositions of both organelles responded in a similar, but not identical, way to temperature change indicated that the system of preference used to established the characteristic ciliary lipid pattern was definitely influenced by the composition of the pool of lipids available for dissemination.

Transfer of Lipids between Microsomes and Cilia. The dynamics of lipid exchange between microsomes and cilia were examined experimentally by two independent approaches. The first study involved analyzing the time course of lipid changes in cilia during the first hours following chilling (Table IV). Unlike the microsomal phospholipids, which by 1 h after chilling exhibited pronounced changes in fatty acid composition toward the pattern of cells fully acclimated to 15 °C (Dickens & Thompson, 1982), the ciliary lipids showed little change 4 h after chilling and displayed the first clear signs of a trend toward the 15 °C pattern only at 8 h following chilling. The frequently noted initial response to chilling, a temporary rise in the level of 16:1 (Nozawa & Kasai, 1978; Martin & Thompson, 1978), was detected to peak in ciliary PE and PC at about 8–12 h after the temperature shift, much later than observed in microsomes (Martin & Thompson, 1978) or whole cell phospholipids (Watanabe et al., 1981).

In a second study of intracellular lipid movement, the rate of specific radioactivity equilibration between microsomal and ciliary lipids was measured after chilling cells prelabeled with [^{14}C]palmitate. The fact that a large differential in specific radioactivity was maintained for 12 h at 15 °C confirmed that lipid exchange between the two organelles was extremely slow, even more sluggish that the rate observed earlier in nonchilled cells (Nozawa & Thompson, 1971b). Despite the lack of evidence for a rapid mixing of lipids from the two organelles, each cell fraction manifested a steady decline in the specific radioactivity of PC with a concurrent rise in AEPL specific radioactivity. This finding is compatible with earlier evidence (Thompson, 1969) which favored an interconversion of these two phospholipids.

The factors responsible for the slowness of change in ciliary lipid composition are not clearly understood. Nevertheless, one may infer that these slow changes are in part responsible for the 15-h period required for cells to resume growth following the 39 to 15 °C shift.

Effects of Lipid Changes on Physical Properties. The various experimental approaches utilized in the present study all reinforced the concept that ciliary membrane lipids were metabolically remote from and poorly accessible to the rapid biochemical alterations triggered in microsomal membranes by chilling. Yet the barriers restraining free movement of lipids between the two compartments did permit a slow and highly selective movement of certain lipids, as evidenced by the gradual change in ciliary lipid composition. The physical properties of ciliary membranes from cells grown at the two

temperatures remained much more similar, as reported by the fluorescent probe DPH, than did the equivalent properties of microsomal membranes (Dickens et al., 1980; Dickens & Thompson, 1981). This is surprising since the cilia from 39 and 15 °C grown cells exhibited relatively larger changes in phospholipid proportions and overall fatty acid composition than did microsomes (Dickens & Thompson, 1982).

The fluidity of the 15 °C ciliary membranes or membrane lipids was greater, as inferred from polarization values measured at a common temperature, than that of 39 °C ciliary membranes of membrane lipids. But the absence in ciliary preparations of the sharp changes in slope (break points) always found in polarization vs. temperature plots of microsomal phospholipids, total lipids, and intact membranes (Dickens & Thompson, 1980, 1981) was unmistakable, especially since microsomal preparations were also being analyzed during this period and could be compared under identical conditions. Some compositional feature of the ciliary phospholipids appeared to favor their highly cooperative reaction to temperature variation, and this uninterrupted physical response persisted even in the low temperature modified lipids. Recent physical studies suggest that the behavior of the ciliary phospholipids is less likely to result from the presence of distinctive polar groups, i.e., ceramide lipids, than from a distinctive pattern of attached hydrocarbon chains (Lentz et al., 1981). The ceramide lipids could, in fact, make a sizable contribution to the observed changes in ciliary membrane physical behavior because of their changing fatty acid composition. More details of sphingolipid involvement, especially as regards the role of hydroxy fatty acids, must await additional studies, some of which are presently under way. Since the present paper emphasizes a comparison of glycerophospholipid characteristics, further details of sphingolipid will be presented elsewhere.

Unlike the situation in microsomes, enough of the cholesterol-like triterpenoid tetrahymanol was present in ciliary total lipids to buffer the effects of temperature on membrane fluidity. This is evident from the comparatively gradual slopes of the ciliary total lipid P vs. T curves (Figure 1A). The moderating effect of tetrahymanol on fluidity change may obviate the need for a rapid dissemination of microsomally altered lipids to the cilia. Whether the reduced concentration of tetrahymanol in cilia of 15 °C grown cells contributes to their increased fluidity must be determined by more detailed experimentation.

Several conclusions can be drawn from the polarization measurements: (1) the ciliary preparations were less fluid than those from microsomes; (2) the detailed physical responses of the two organelles to temperature change were significantly different; and (3) although low temperature induced a definite fluidization of ciliary lipids the changes in ciliary membrane properties were less pronounced than were those induced in microsomal membranes.

Detailed studies of the microsomal lipid molecular species composition (Dickens & Thompson, 1982) indicated that intermolecular rearrangements of fatty acids may have had much to do with membrane fluidity changes at low temperature in that organelle. The following paper (Ramesha et al., 1982) describes a similar phospholipid molecular species analysis of ciliary phospholipids from 39 and 15 °C grown *Tetrahymena*. A comparison of the phospholipid molecular species analysis of ciliary phospholipids from 39 and 15 °C grown *Tetrahymena* with the molecular species found in microsomes under equivalent conditions sheds further light on the metabolic interrelationships between the two organelles.

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