

**TETRAHYMENA STRIVES TO MAINTAIN THE FLUIDITY
INTERRELATIONSHIPS OF ALL ITS MEMBRANES CONSTANT**
Electron Microscope Evidence

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ABSTRACT

When cells of *Tetrahymena pyriformis*, strain NT-1, were chilled from their growth temperature of 39.5°C to lower temperatures, the plasma membrane, outer alveolar, nuclear, outer mitochondrial, food vacuolar, and endoplasmic reticulum membranes each responded in a fashion quite characteristic of the membrane type. In most cases a distinctive rearrangement of intramembrane particles, as discerned by freeze-fracture electron microscopy, began abruptly at a definitive temperature. By comparing the freeze-fracture patterns of membranes in cells grown at 39.5, 27, and 15°C, it was shown that the initial particle rearrangement in a given membrane always occurred at a fixed number of degrees below the growth temperature of the cell. Gradual chilling of a cell grown at constant temperature induced these membrane changes first in the outer alveolar membrane, then, in order of decreasing response to temperature, in the endoplasmic reticulum, outer mitochondrial membrane, nuclear envelope, and vacuolar membrane. The normally stable relationships between the physical properties of the several membrane types could in some cases be reversed, but only temporarily, by fatty acid supplementation or during the initial phases of acclimation to growth at a different temperature. The system provides a unique opportunity to study the effects of environmental change upon the physical properties of several functionally distinct but metabolically interrelated membranes within a single cell.

The protozoan *Tetrahymena pyriformis* is an excellent test organism for studying the mechanism of membrane change during temperature acclimation (9, 13, 28). Using the thermotolerant NT-1 strain, Fukushima et al. (3) showed that several specific membranes of cells acclimated to 15°C differed from those of 39.5°C cells by having significantly more polyunsaturated fatty acids and a different distribution of phospholipids. The same membranes isolated from cells grown at an intermediate temperature (26°C) were only slightly different from the 15°C pattern. These findings extended the earlier work of Wunderlich and

Ronai (27), who showed a similar tendency in smooth microsomal membranes of a nonthermotolerant *Tetrahymena* strain.

We wondered how this nonlinear change of membrane phospholipid fatty acid unsaturation with temperature of acclimation was related to the physical properties of the affected membranes. Accordingly, we carried out an analysis by freeze-fracture electron microscopy to monitor changes in membrane particle distribution on the hydrophobic faces revealed by cleaving the membrane lipid bilayers. Characteristic temperature-induced rearrangements of membrane particles have been

reported for several *Tetrahymena* membranes (21, 29, 30), and such changes have been related to changing fluidity, both in *Tetrahymena* (3, 9, 13, 28) and in other cells (6, 8, 11, 20, 26, 31). The results described in the present communication suggest that *Tetrahymena* maintains each of its several membrane types at a characteristic fluidity regardless of the growth temperature.

MATERIALS AND METHODS

Tetrahymena pyriformis, strain NT-1 (3), was grown in 200-ml shake cultures of enriched proteose peptone medium at 39.5, 27, and 15°C as previously described (22), until the cells reached a density of $7-8 \times 10^4$ /ml.

30-ml aliquots of a culture were transferred to 100-ml Erlenmeyer flasks which had been equilibrated to the growth temperature of that culture. Each aliquot was then chilled over a 4-min period to an appropriate temperature (33, 30, 27, 24, 21, 18, 15, 12, 9, 5 and 0.5°C were used for 39.5°C-cells), and incubated for 5 min before fixation for electron microscopy. In control experiments, identical results were obtained (except for the plasma membrane, see below) using cells chilled over a period of 1 or 2 min rather than 4 min or in cells incubated at the desired final temperature for 2, 15, or 30 min before fixation rather than the standard 5-min period. The glutaraldehyde fixative has been shown to fix membrane particles permanently in place so that further temperature change or manipulations have no effect on the pattern of distribution (11).

In those cases where linoleic acid (*cis,cis*-9,12-octadecadienoic acid [18:2]) (Supelco Inc., Bellefonte, Pa.) was used for supplementing the growth medium, it was prepared as a sonicated emulsion of 3.2 μ mol acid in 3 ml of inorganic medium (5). The supplement was added dropwise into a 200-ml culture with shaking over a 10-min period. 30 ml of the culture was subjected to fixation for electron microscopy after incubation with linoleic acid for 90 or 345 min at 39.5°C.

For freeze-fracture electron microscopy, the 30-ml specimen cultures described above were poured quickly into an equal volume of 2% glutaraldehyde solution buffered with sodium phosphate buffer (0.1 M, pH 7.2). The mixture was then incubated for 15-20 min at the appropriate temperature, care being taken to control the temperature to within $\pm 0.5^\circ\text{C}$ during the fixation. The fixed cells were then immersed in increasing concentrations of glycerol, terminating with a 24-48 h incubation with 30% glycerol. A drop of pelleted cells was placed onto a gold disk and frozen in liquid Freon 22 held close to its freezing point in a bath of liquid N_2 . Frozen cells were cleaved in a Balzers BA, 360 M device (H. Balzers, BA, 360 M, Fürstentum, Liechtenstein) and shadowed with platinum and carbon at -110°C without etching. Replicas were cleaned in 40% chromic acid at 50°C for 2 h, rinsed three times with double-distilled water, and then examined with a Hitachi HS-8 electron microscope at 50 kv.

The particle density index (PDI) of the outer alveolar membrane was determined as previously described (13). After placing a grid over enlarged micrographs to simulate a 200×200 nm area over regions of the highest particle density, the enclosed particles were counted to estimate the number of particles per square micrometer. 35-50 areas on 7-10 different replicas were counted for every sample fixed at a particular temperature. The PDI was obtained by assigning the number of particles per square micrometer of 39.5°C-fixed membrane (representing a random particle distribution) a value of *a* and that of 0.5°C-fixed membrane (representing maximum aggregation) a value of *b*. In our previous paper (13), *a* and *b* were calculated as 388 and 2,633, respectively. The PDI was then calculated by the following equation, with *Y* as the particle density of a membrane fixed at an intermediate temperature.

$$\text{PDI \%} = \frac{Y - a}{b - a} \times 100 = \frac{Y - 388}{2,633 - 388} \times 100$$

RESULTS

Indications of Particle Rearrangements

A number of reports have described details of particle distribution in various functionally different membranes of *Tetrahymena* as revealed by freeze-fracture electron microscopy (17-19, 29). Distinctive patterns of low temperature-induced particle aggregation were first observed by Speth and Wunderlich (21). It was later shown that the aggregation response exhibited by the alveolar membrane did not occur in the endoplasmic reticulum (28). There, the appearance of particle-free domains was not accompanied by an increase in particle density in the adjacent regions of the bilayer. A movement of proteins perpendicular to the plane of the membrane was postulated to explain the disappearance of particles with decreasing temperature.

We have confirmed the findings of Wunderlich et al. (28) using the thermotolerant strain NT-1 of *Tetrahymena*. Because strain NT-1 can be grown at an elevated temperature (39.5°C), we were also able to determine the mode of particle redistribution in some of the more fluid membranes by rapidly chilling the cells over an unusually large temperature range.

By removing aliquots from a culture growing at constant temperature and chilling each to one of several lower temperatures, the initiation point of particle redistribution could be established for each membrane. Figs. 1-6 illustrate the freeze-fracture appearance of the plasma membrane, outer alveolar, endoplasmic reticulum, nuclear, outer mitochondrial, and food vacuolar mem-

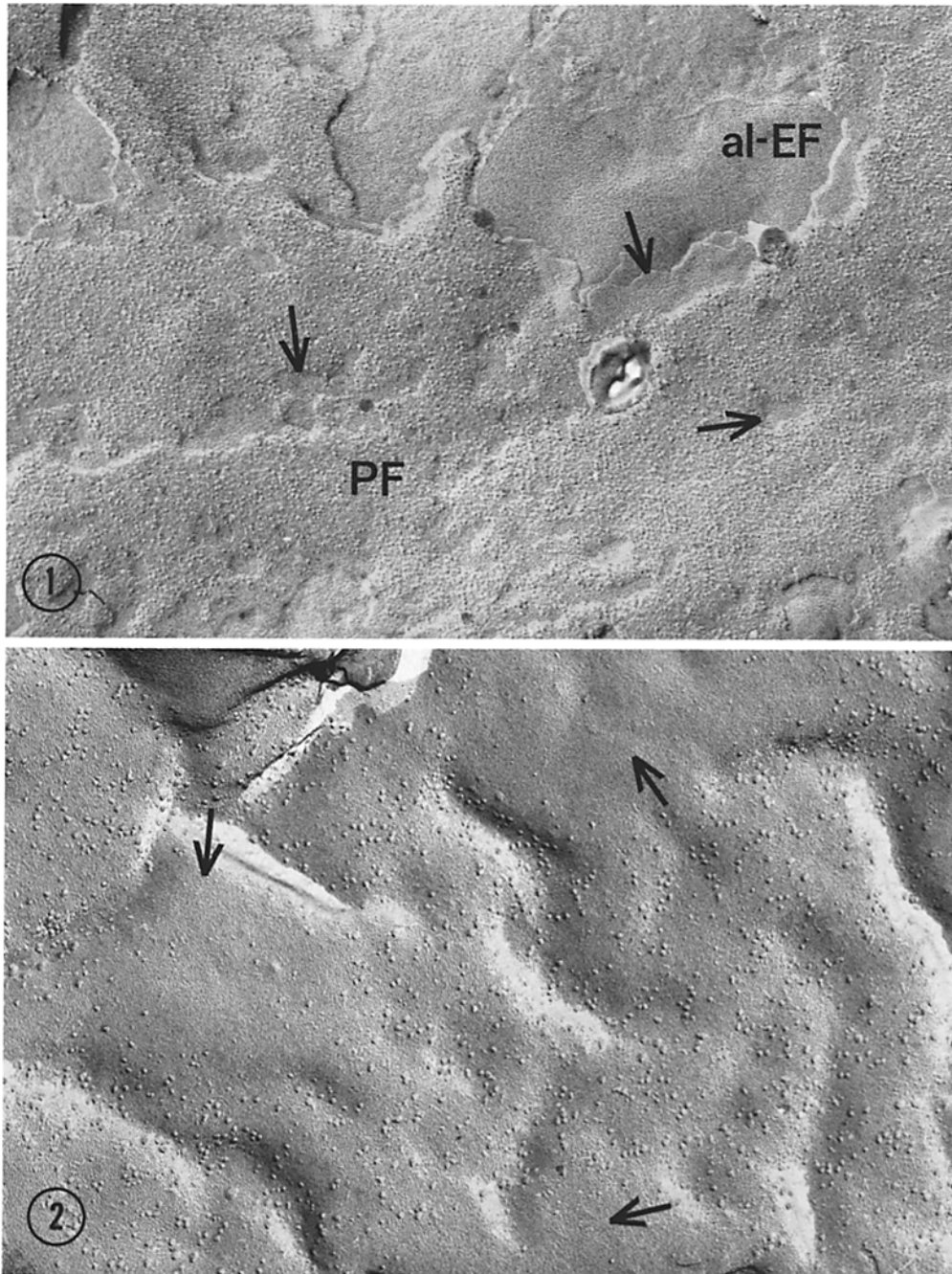


FIGURE 1 The effects of rapid chilling from 39.5°C to 5°C on the plasma membrane. Particle-free and depressed areas (arrows) are produced on the inner face (PF) of the fractured plasma membranes. Shadowing directions are from the bottom to the top in all electron micrographs presented in this article. *al-EF*, EF face of the outer alveolar membrane. $\times 63,000$.

FIGURE 2 Particle rearrangement seen on the outer face (PF) of the fractured outer alveolar membrane viewed from the inside of the alveolar sac. Particle-free areas (arrows), apparently due to clustering of crystallized lipid molecules, are surrounded by areas of randomly distributed particles. Particle redistribution first starts when the cells are chilled to 33°C. This figure represents 39.5°C-grown cells chilled to 30°C. The particle density in the particle-rich regions continues to increase as the temperature decreases (see Fig. 7). The particles ($\sim 115 \text{ \AA}$) are larger than those of the plasma membrane ($\sim 75 \text{ \AA}$). $\times 63,000$.

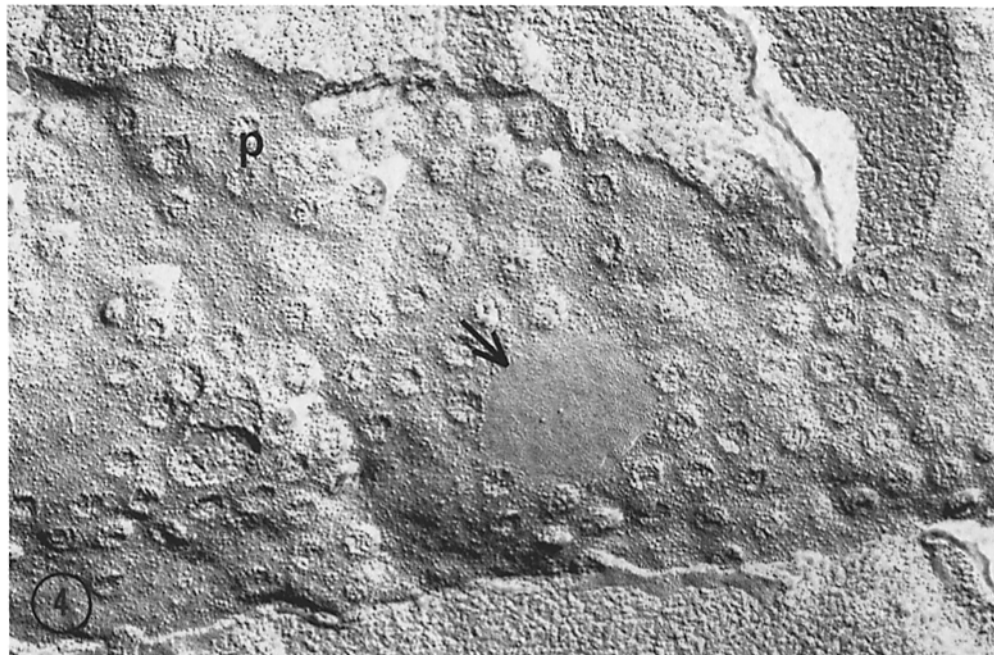
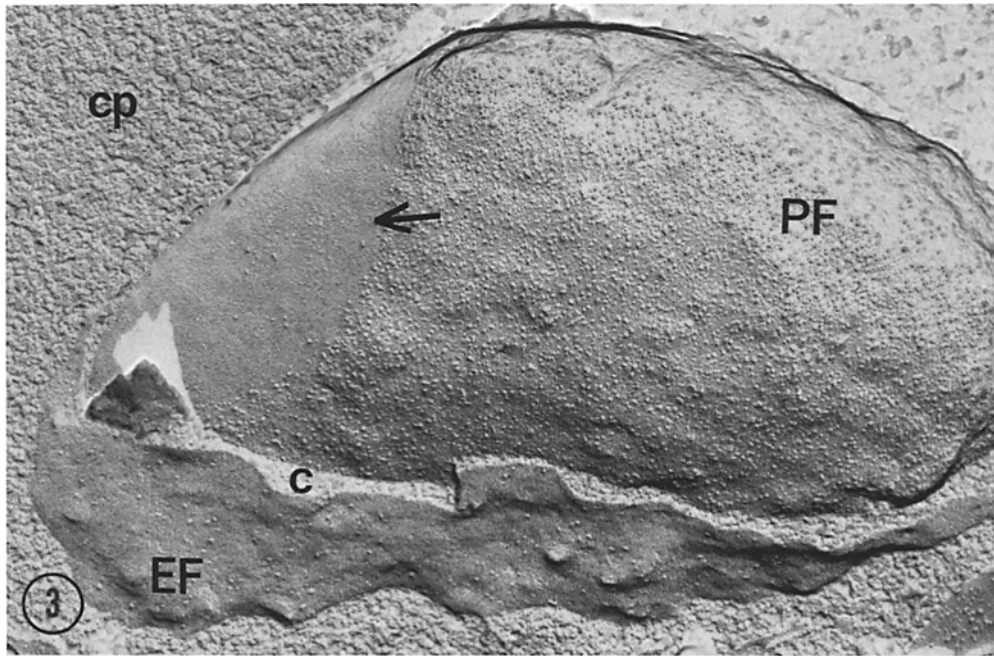


FIGURE 3 The effects on the endoplasmic reticulum membranes of rapidly chilling 39.5°C-grown cells to 18°C. PF-face of the fractured membrane (seen from the cisterna toward the cytoplasmic side) reveals a large particle-free area (arrow) separated from the randomly particulated areas. These particles display a heterogeneity in size (50-100 Å) and show, in replicas prepared at several temperatures, no tendency to aggregate. (CP) cytoplasm, (C) cisterna of endoplasmic reticulum, (EF) the cisternal side of the membrane viewed from the cytoplasmic side. × 63,000.

FIGURE 4 The effects on the nuclear membrane of rapidly chilling 39.5°C-grown cells to 15°C. Circular, particle-free, smooth areas are produced on both fracture faces. This micrograph shows a smooth area (arrow) produced on the PF face of the inner nuclear membrane. Nuclear pores (P), which are seen as crater-like, circular depressions, have disappeared from the phase separated smooth area. × 63,000.

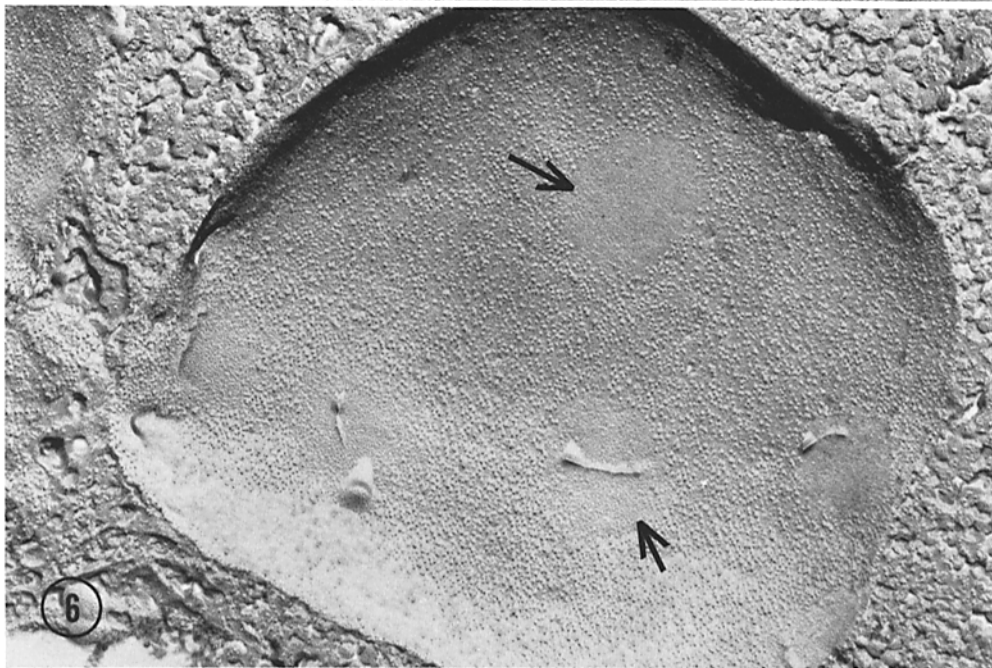
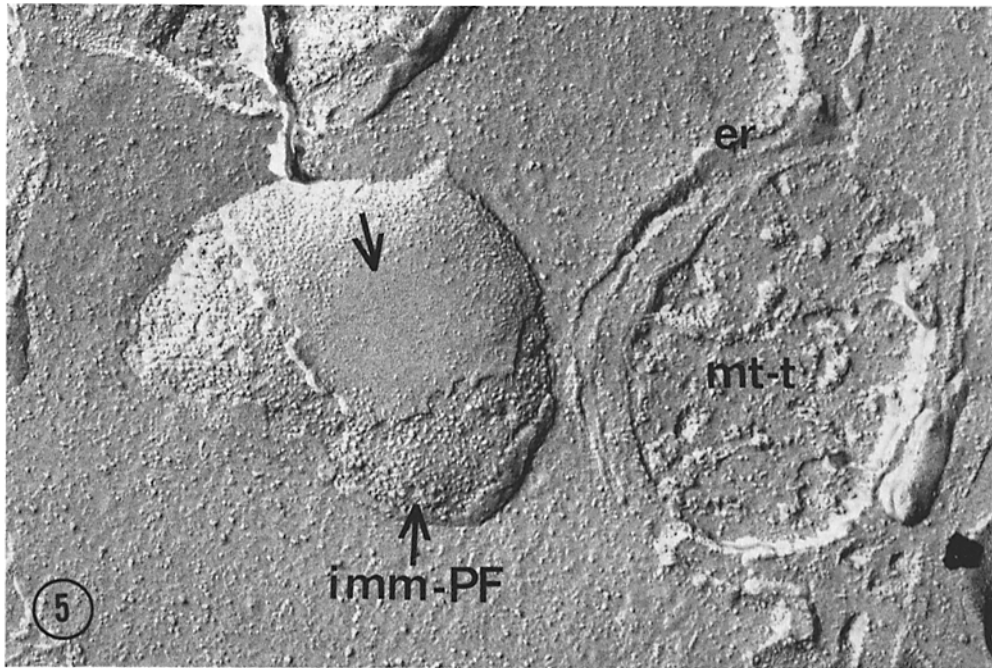


FIGURE 5 A particle-free area (arrow) produced on the EF face of the outer mitochondrial membrane. These alterations are induced on both fracture faces of the outer mitochondrial membrane when 39.5°C-grown cells are chilled below 21°C. (*imm-PF*) inner fracture face of the inner mitochondrial membranes viewed from the cytoplasmic side toward the mitochondrial inner membrane; (*mt-t*) transverse cleaved face of mitochondrion; (*er*) endoplasmic reticulum. $\times 63,000$.

FIGURE 6 Particle rearrangement occurring on the vacuolar membrane of 39.5°C-grown cells chilled to 5°C. Particle-free, smooth, circular areas are seen on the PF-face of the fractured membrane. The particles are homogeneous in size (about 75 Å) and show no detectable aggregation. In some separated areas, lines are seen. $\times 63,000$.

branes, respectively, of 39.5°C-grown cells at temperatures just below the point at which particle-free domains first appear. Membrane-core alterations caused by rapid chilling of cell samples to several predetermined lower temperatures could be divided into three types: (a) The induction of particle-free areas and simultaneous particle aggregation, as seen on the outer fracture face (PF) of the outer alveolar membrane (Fig. 2). Pronounced aggregation due to lateral particle movement has been quantified in an earlier report (13). (b) The appearance of circular particle-free areas without particle aggregation, as seen on both the inner and outer fracture faces (EF and PF) of the endoplasmic reticulum (Fig. 3), the nuclear (Fig. 4) and the outer mitochondrial (Fig. 5) membranes, and on the outer fracture face (PF) of vacuolar membranes (Fig. 6). (c) The appearance of depressed, particle-free areas on the inner fracture face (PF) of plasma membrane (Fig. 1). All of these alterations were reversed when the cells were warmed to the original growth temperature. No alterations were induced on the ciliary membranes at any fixing temperature above 0°C that we used here. Apart from the two alveolar membranes, the temperature-induced appearance of particle-free areas in the different membranes was not accompanied by significant particle aggregation, even at fixation temperatures near 0°C.

In addition to the 39.5°C cells, cells grown at 27 and 15°C were examined. The patterns observed were almost identical to the findings illustrated in Figs. 1-6, except for changes in the temperature at which the alteration of particle distribution was first detected.

Temperature Control of Particle Rearrangements

Each membrane type had its own characteristic temperature at which signs of phase separation could be detected. Table I lists these temperatures for cells grown many generations at 39.5°C. The surface membranes, represented by the plasma membrane and the ciliary membrane, are quite resistant to particle rearrangement. This may well be due to the elevated level of tetrahymanol reported to be present there (23). On the other hand, the outer alveolar membrane, which lies immediately below the plasma membrane (29), is the structure most susceptible to temperature-induced particle movement. The remaining intracellular organelles are visibly altered over the 24-12°C range.

In the alveolar membranes, which undergo an aggregation of particles due to lateral movement, this movement can be quantified (13). In Fig. 7, we have used freeze-fracture data for the outer alveolar membrane of 39.5- and 15°C-grown cells reported earlier (13) and for 27°C-grown

TABLE I
Observation of Particle-Free Regions in Different Organellar Membranes of *T. pyriformis* Cells Grown at 39.5°C as Visualized by Freeze-Fracture Electron Microscopy*

Temperature of fixation	Plasma membrane	Outer alveolar membrane	Inner alveolar membrane	Endoplasmic reticulum	Nuclear membrane	Vacuolar membrane	Mitochondrial membrane	Small vesicle membrane	Ciliary membrane
39.5	-	-	-	-	-	-	-	-	-
33	-	+	-	-	-	-	-	-	-
30	-	+	-	-	-	-	-	-	-
27	-	+	-	-	-	-	-	-	-
24	-	++	+	+	-	-	-	+	-
21	-	++	+	+	-	-	+	+	-
18	-	++	+	+	+	-	+	+	-
15	+?‡	++	+	+	+	-	+	+	-
12	+?‡	++	+	+	+	+	+	+	-
9	+	++	+	+	+	+	+	+	-
5	+	++	+	+	+	+	+	+	-
0	+	++	+	+	+	+	+	+	-

* Whole cells were fixed for microscopy after a brief equilibration at one of the indicated temperatures. - = no change from normal particle distribution at 39.5°C; + = appearance of particle-free domains; ++ = pronounced particle aggregation.

‡ Particle-free areas not seen unless cells were held at this temperature for a longer time.

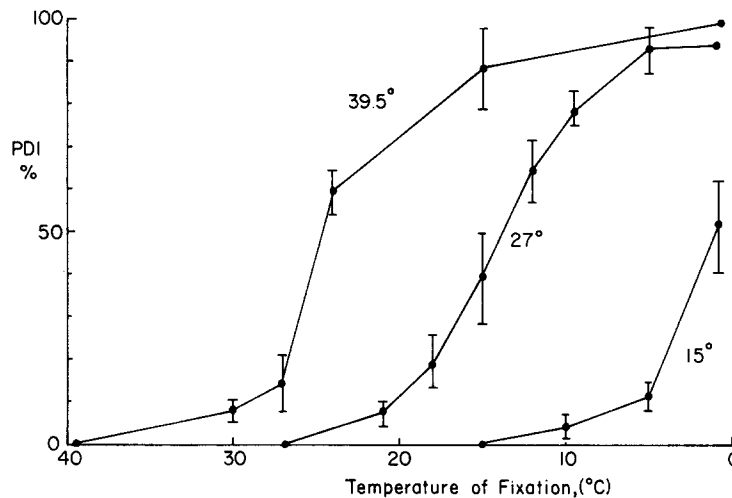


FIGURE 7 The influence of temperature upon particle aggregation in the outer alveolar membrane of cells grown at 39.5, 27, and 15°C. The particle density index (PDI) was determined as described in Materials and Methods.

cells to plot the PDI (see Materials and Methods), against temperature. According to this formulation, a PDI of 0% means no aggregation while a PDI of 100% means maximal aggregation. Cells grown at the three temperatures each initiate particle aggregation and reach the midpoint of aggregation (PDI of 50%) at nearly the same number of degrees below their growth temperature. Thus, the temperature of PDI 50% is 15.5, 13.5 and 14°C below growth temperature for 39.5, 27, and 15°C cells, respectively.

The striking regularity is also found in membranes where particle aggregation itself is not observed. The temperatures causing the first appearance of particle-free regions in various membranes can be used as indicators of physical change. These initiation temperatures are plotted against growth temperature in Fig. 8. While measurements below 0°C in cryoprotectants were not attempted, all evidence attainable with cells in growth medium suggested a linear decrease in phase separation with temperature.

Effect of Lipid Supplementation on Membrane Phase Transition

The uniform relationships between the particle rearrangement initiation temperatures in various membranes can be perturbed by supplementing the growth medium with fatty acids. We have recently described the rates at which cells growing at 39.5°C incorporate linoleic acid (18:2) into

their phospholipids (9). It was clear from cell fractionation studies that the 18:2 was initially incorporated into the endoplasmic reticulum phospholipids, and that with time the abnormally high degree of unsaturation there was gradually shifted into other organelles. In Fig. 9 it can be seen that the freeze-fracture evidence for membrane particle movement mirrors the findings of altered lipid composition. Shortly after the massive uptake of 18:2, the internal membranes, particularly the endoplasmic reticulum and the nuclear envelope, exhibited particle-free regions at much lower than normal temperatures. There was no evidence of heterogeneous fluidity within the endoplasmic reticulum at the time when the fed 18:2 was localized there (2-h point), suggesting a uniform distribution of the highly unsaturated phospholipids. However, we cannot rule out the possibility that selective fracturing concealed regions of higher particle density.

It was of particular interest to observe an apparently unidirectional movement of the superfluid lipids outwards into the alveolar membrane as a "pulse", resulting in a reversal in the normal relationship between the midpoint of outer alveolar membrane phase separation and the initiation of phase separation of the endoplasmic reticulum (Fig. 9, 5.75 h). By this time, the internal membranes had returned to their original physical state while the outer alveolar membrane was abnormally fluid.

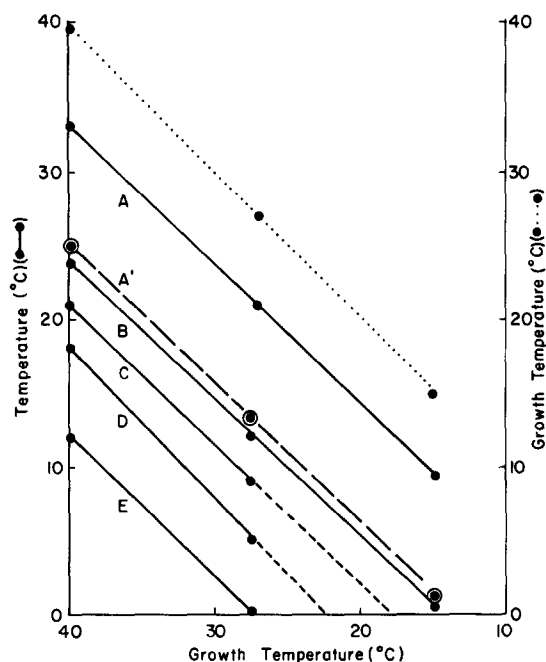


FIGURE 8 The temperatures (—●—) at which particle-free regions first appear in several membranes of cells grown at 39.5, 27, and 15°C before rapid chilling and glutaraldehyde fixation at temperature intervals of approximately 3°C (see Materials and Methods). The fixed cells were analyzed by freeze-fracture electron microscopy. (A) outer alveolar membrane, (A') midpoint (50% PDI) of particle aggregation in outer alveolar membrane; (B) endoplasmic reticulum; (C) outer mitochondrial membrane; (D) nuclear envelope, (E) vacuolar membrane. The upper line (....●....) and right axis simply indicate the growth temperatures examined.

Effect of Temperature Acclimation Upon Membrane Particle Rearrangements

The changes in lipid composition during the acclimation of 39.5°C-grown cells to growth at 15°C have recently been described (13). The gradual fluidizing effect on the outer alveolar membrane caused by increased fatty acid desaturation was followed by freeze-fracture electron microscopy in that paper. We have now examined in some detail the changes sustained by other membranes during the acclimation period.

Fig. 10 illustrates that the fluidity of most internal membranes appears to have increased faster than that of the outer alveolar membrane during the first 4 h of the acclimation period. The vacuolar membrane was an exception. Quite unexpectedly, however, no further fluidity increase

was indicated for endoplasmic reticulum, mitochondrial outer membranes and nuclear membranes during the next 4-h period. On the other hand, the outer alveolar membrane, which was the only one still showing particle-free domains after 4 h at 15°C, continued to decline in aggregation, reaching the level found in long-term 15°C-grown cells by 8 h following the shift. Again, the pattern suggests that the more fluid phospholipids formed (in this case from endogenously produced fatty acids) were transported outward into surface membranes. It was of interest that the freeze-fracture-inferred increase in endoplasmic reticulum fluidity did not continue during the period 4–8 h after the temperature shift down to 15°C.

DISCUSSION

By growing strain NT-1 of *Tetrahymena* at its upper temperature extreme, we were able to demonstrate that the membranes of each organ-

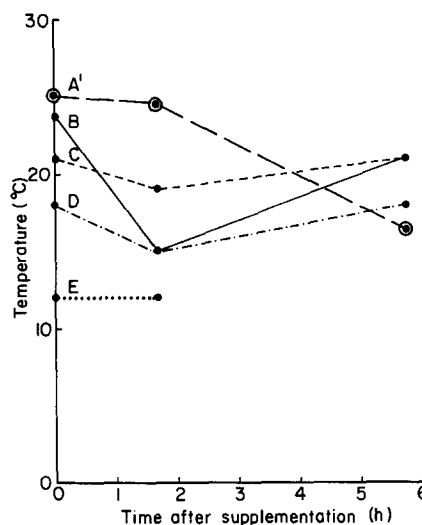


FIGURE 9 The effects of linoleic acid (18:2) supplementation (see Materials and Methods) upon membrane particle rearrangements in 39.5°C-grown cells. The abscissa represents the time, after beginning the addition of 18:2, at which cells were chilled to various temperatures (shown on ordinate) for fixation before the freeze-fracture treatment. A' = midpoint (50% PDI) of particle aggregation in the outer alveolar membrane. The other lines represent the temperatures at which particle-free regions first appeared in endoplasmic reticulum (B), mitochondrial outer membrane (C), nuclear envelope (D), and vacuolar membrane (E).

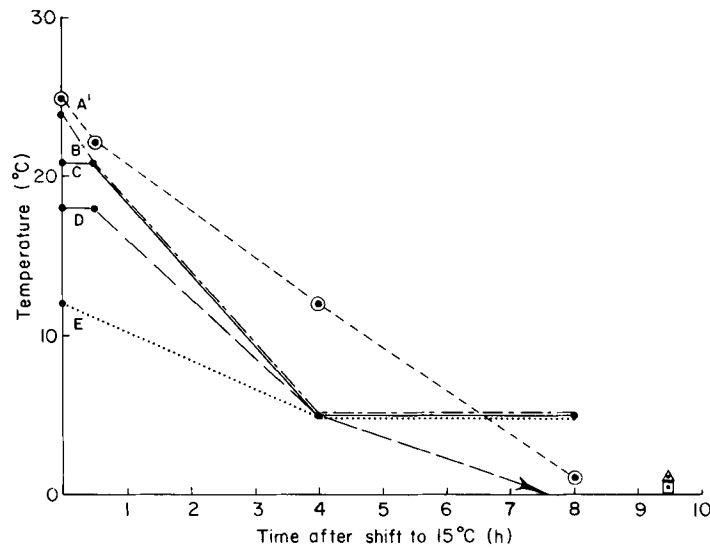


FIGURE 10 Changes in the particle rearrangement temperatures of 39.5°C-grown cell membranes during acclimation to 15°, (A') = midpoint (50% PDI) of particle aggregation in outer alveolar membrane. The other lines represent the temperatures at which particle-free regions first appeared in endoplasmic reticulum (B), mitochondrial outer membrane (C), nuclear envelope (D), and vacuolar membrane (E).

elle respond to chilling in a characteristic and different fashion. The particle distribution in some membranes was altered by only slight chilling while other membranes required a temperature drop of 28°C before rearrangement took place. Only one membrane, that sheathing the cilia, retained its normal particle distribution after even a 38°C reduction in temperature. In most cases, the morphological pattern resulting from chilling was identical to that previously noted in *Tetrahymena* by Wunderlich et al. (21, 30, 28). The earlier workers, using the less thermotolerant GL strain (30), could detect no temperature effect in vacuolar membranes. We found a distinctive occurrence of circular, particle-free, smooth areas on the vacuolar membrane PF face in 39.5°C-grown cells chilled rapidly to 12°C or lower. Cells grown at 27°C showed a low frequency of particle-free areas at 0°C, identifying that as the temperature of incipient particle rearrangement in the vacuolar membranes. No sign of particle rearrangement was detected in vacuoles of 15°C-grown cells, even at 0°C.

The most valuable new contribution of our present work is to establish a quantitative basis for measuring the effect of temperature on particle redistribution in the various organellar membranes. The information thus obtained is complementary to quantitative measurements of fluidity

by physical chemical techniques, furnishing a different and entirely independent assessment of membrane changes due to temperature.

The diversity of the freeze-fracture patterns resulting from falling temperature (Figs. 1-6) would suggest that the nature of the integral proteins in each distinct membrane and the interaction of those proteins with other elements of the cell are important in determining the type of pattern observed. The profound influence of peripheral proteins on the lateral movement of erythrocyte membrane integral proteins has been demonstrated (1). However, the surprisingly uniform quantitative relationships among different membranes, as illustrated by Fig. 8, lead us to believe that the factor triggering the pronounced temperature-induced rearrangement of membrane particles is in all cases a phase separation in the lipid bilayers. There is much evidence in the literature to support this contention (4, 6, 8, 11, 12, 16, 20, 25, 26), including some evidence gained from the study of *Tetrahymena* membranes (21, 30, 28). In the latter case, the appearance of smooth areas in freeze-fractured *Tetrahymena* microsomes with decreasing temperature was closely correlated with discontinuities in the fluorescence intensity of 8-anilino-1-naphthalenesulfonate, in the pattern of change in electron spin resonance (ESR) spectra, and in

the activity of glucose-6-phosphatase (28). Although no sharp phase transition could be detected in extracted lipids, it was apparent that the altered particle orientation in freeze-fracture membrane replicas resulted from the temperature-induced separation of lipids into environments of differing fluidity.

Nozawa et al. (15) compared the relative fluidity of several *T. pyriformis*, strain WH-14, organelles, including pellicles, ciliary membranes, and microsomes, using electron spin resonance spectrometry. A relatively uniform increase was found in the fluidity of all three organelles (as measured at a standard temperature) with decreasing growth temperature. Pellicle fluidity responded somewhat more than did the fluidity of cilia or microsomes. At all temperatures, the fluidity of ciliary membranes was relatively lower than that of the other membranes isolated from the same cells. Microsomes exhibited the highest fluidity.

These ESR estimates of relative fluidity differences among different membranes would not seem to agree with our present findings. For example, the low fluidity of ciliary membranes might be expected to result in phase separation after only relatively slight chilling, but the ciliary membranes were in fact the most resistant to temperature modification in our study. The apparent conflict in this instance may be explained by the occurrence in ciliary membranes of a six-fold higher ratio of tetrahymanol to phospholipid than is found in microsomes (22). The strong damping effect of the cholesterol-like tetrahymanol molecule upon fluidity change is probably responsible for the absence of an apparent phase separation in ciliary membranes. Detailed analyses of membrane fluidity by fluorescence polarization techniques are currently underway in our laboratory.¹

Our data and those described above lead us to three interpretations. (a) Temperature-induced membrane particle rearrangement is probably an indication of lipid phase separation. (b) Comparisons of freeze-fracture-indicated "phase separation" temperatures between functionally-different membranes are not valid measurements of relative fluidity differences at a particular growth temperature. Fluidity and rates of temperature-induced fluidity change are very dependent upon factors such as lipid composition, the amount and

types of bound cations, the pH, and the membrane protein complement (2, 7, 12, 15, 16). Cases are known where these factors or the interaction of integral proteins with a meshlike system of underlying structural components, such as exists in the *Tetrahymena* pellicle (24), can induce membrane particle aggregation without a change in lipid bilayer fluidity (1). Therefore, a certain *Tetrahymena* membrane showing a completely random particle distribution might actually be less fluid than another membrane exhibiting particle aggregation. (c) Comparisons of freeze-fracture-indicated phase separation temperatures between the same membrane types in cells grown at different temperatures probably are valid measurements of relative fluidity. Our work (13) showed that during the temperature acclimation period the major lipid changes are in the degree of fatty acid unsaturation of phospholipids. There is little change in phospholipid distribution during this period, and earlier work (3) showed tetrahymanol levels to remain constant. Drastic alterations in protein composition or bound cation level in a particular membrane are unlikely to result from temperature-induced lipid changes of this type, and therefore these factors should not be considered as important variables.

We conclude, therefore, that data of the type plotted in Fig. 8 provide an accurate estimate of temperature-induced fluidity changes in a given type of membrane. The fact that the relative differences between functionally distinct membranes remain constant (albeit quantitatively unknown) is of fundamental importance in establishing the principles of membrane interrelationships.

Why should *Tetrahymena* maintain these constant fluidity differentials between its membranes? There is ample evidence to suggest that enzymes function less efficiently if membrane fluidity is reduced until the lipids of their environment are in the gel rather than the liquid-crystalline state (10, 14). However, there seem to be few marked changes in the rate of enzymatic reactions in the temperature range above the phase transition. One case in which enzymatic activity was altered due to fluidity changes wholly within the liquid-crystalline state was observed in *Tetrahymena* shifted from a growth temperature of 15 to 39.5°C. Here an increase in fluidity beyond the optimal level caused a reduction in fatty acid desaturase activity (13). Although the reduction in desaturase activity

¹ C. E. Martin, unpublished observations.

brought about by this shift was appreciable, the rise in fluidity which produced it was also unusually large. The significance of such a regulatory mechanism in nature is not known.

Rapid changes in temperature or massive uptake of unsaturated fatty acids can upset the normal membrane fluidity interrelationships. We have recently shown that the incorporation of exogenously supplied linoleic acid to 39.5°C-grown cells reduces the action of fatty acid desaturases (9). Conversely, desaturase activity increases when 39.5°C cells are chilled to 15°C (13). In both cases, as illustrated in Figs. 9 and 10, perturbations of the steady-state fluidity interrelationships occur. From previous studies (9, 13), we can understand why the initial fluidity alterations are most pronounced in the endoplasmic reticulum. Changes in the assortment of fatty acids made and incorporated into phospholipids there permit the cell to gradually restore optimum fluidity to membranes throughout the cell. In the case of 39.5°C cells fed linoleate, the freeze-fracture data (Fig. 9) indicate that it is the endoplasmic reticulum which most sensitively reflects the initial impact of the linoleate and also the eventual response leading ultimately to a restoration of the normal patterns. Likewise, the endoplasmic reticulum is most responsive to reduced temperature (Fig. 10). Here, for reasons not presently understood, the temperature of apparent initial phase separation for the endoplasmic reticulum, mitochondrial outer membrane, and nuclear membrane did not continue to fall uniformly towards the steady state 15°C values. There was no change in apparent fluidity during the period 4–8 h after the shift to 15°C. The exact time required for total restoration of normal 15°C-fluidity levels throughout the cell has not been determined. It is clear, however, that growth of cells at a new temperature, such as 15°C, for only a few generations restores the intracellular fluidity relationships to those of cells fully acclimated to that temperature. The very fact that the fixed hierarchy of differential membrane temperature responses (Fig. 8) is quickly restored during the acclimation period indicates that the physical state of each membrane is carefully regulated.

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