ADAPTIVE LOWERING OF THE LIPID CLUSTERING TEMPERATURE WITHIN *TETRAHYMENA* MEMBRANES

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1. Introduction

Variations in temperature can effect dramatic changes in structural and functional features of biomembranes. For instance, freeze-etch electron microscopy has recently revealed a lateral separation of membrane components directly within the membrane cores of the endoplasmic reticulum in cells of the unicellular eukaryote *Tetrahymena* upon lowering these cells' environmental temperature from that optimal for growth at 28°C to below 18–16°C [1]. At this temperature range, a discontinuity is also detected in the activity of the endoplasmic reticulum marker enzyme, glucose 6-phosphatase, which is bound to the smooth microsomal membranes isolated from these 28° C-*Tetrahymena* cells [1]. Different physical methods such as electron spin resonance, calorimetry, fluorescence probing, and proton nuclear magnetic resonance have indicated that these thermotropic membrane changes are very probably induced by a clustering of 'rigid' liquid crystalline membrane lipids ([1] cf. also [2]) rather than by a true liquid crystalline ⇌ crystalline lipid phase transition which causes such membrane changes in other cells, e.g. [3–6]. The present paper provides evidence that *Tetrahymena* cells grown at 18°C have adaptively shifted the temperature of the lipid clustering within endoplasmic reticulum membranes down to ∿ 12°C by replacing the more saturated with more unsaturated membrane phospholipid species.

2. Materials and methods

Cell Static cultures of the ciliate protozoan *Tetrahymena pyriformis* GL were axenically grown at 18°C in the logarithmic growth phase (∿20 000 cells/ml) in a medium consisting of 0.75% proteose peptone, 0.75% yeast extract, 1.5% glucose, 0.1 mM ferric citrate, 0.05 mM $CaCl_2$, and 1 mM $MgSO_4$.

Freeze-etch electron microscopy. In three experiments, a *Tetrahymena* culture was divided into several aliquots, which were equilibrated for 10 min at 18°C, 15°C, 11.5°C, 8°C, and 5°C. At these temperatures, the cells were fixed with 2% glutaraldehyde buffered with 0.05 N Na-cacodylate (pH 7.2). After 10 min, the cells were twice washed with the cacodylate buffer and stepwise glycerinated up to 25% for 2 hr, before they were frozen on cardboard disks in Freon 22 cooled by liquid nitrogen. Fracturing, etching (1 min at–100°C) and replication were carried out in a Balzers freeze-etch device model BA 260M. The replicas were studied in a Siemens Elmiskop Ia.

Membrane isolation. The light smooth microsomal fraction containing only smooth-surfaced membrane vesicles was isolated on step sucrose gradients as described recently [7].

Glucose 6-phosphatase. This enzyme was determined at V_{max} in the membrane vesicles fraction as a function of temperature as described previously [1].

Lipid analysis. Total lipids were extracted from the membrane vesicles with chloroform/methanol (2:1) at 0–4°C according to Folch et al. [8]. Polar and neutral lipids were separated, identified and quantified by two- and one-dimensional thin-layer chromatography, respectively, as described recently [7]. The fatty acids of the total lipids, polar lipids and neutral lipids were saponified, methylated and identified in a Varian gas chromatograph as described previously [7].

3. Results

3.1. *Thermotropic membrane response*

At cleavage, freeze-etch electron microscopy exposes two faces of the hydrophobic core of the endoplasmic reticulum membranes in *Tetrahymena* [9,10]. At 18°C and 15°C, the PF-faces (i.e. the faces bordering the cytoplasm) are studded with numerous, uniformly distributed particles, ∿ 7.5 nm in diameter (fig.1a). The opposite EF-faces (i.e. the faces oriented to the cisternal space) show only some particles and numerous depressions (fig.1a). At 11.5°C, however, about 40% of both fracture faces reveal particle-depleted areas (cf. fig.1b). These can be regularly seen in *Tetrahymena* cells equilibrated at 8°C. At 5°C, these smooth areas have largely expanded and can reach diameters of up to 1 μm (fig.1b).

The temperature range of the emergence of particle-depleted areas is the same as that (∿12°C), where the activity of the glucose 6-phosphatase associated with the smooth microsomal membrane vesicles shows a discontinuity (fig.2). Above and below this discontinuity the activation energies lie in the range of ∿ 6 Kcal/mol and ∿ 10 Kcal/mol.

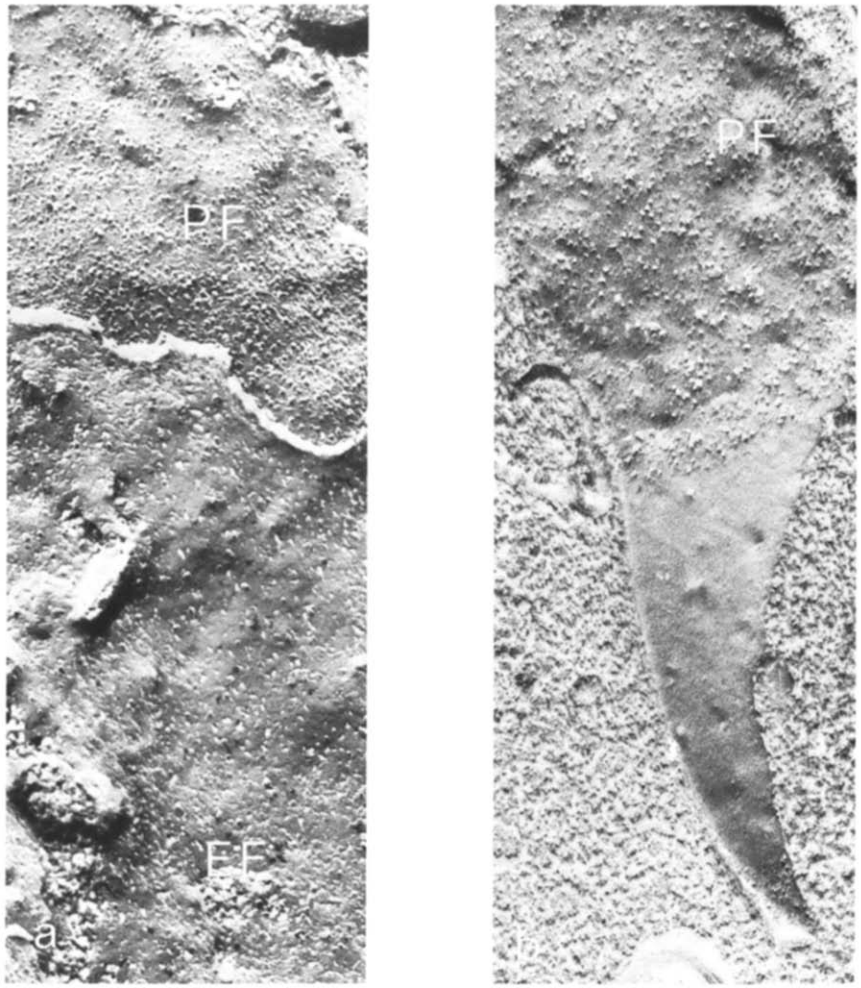

Fig.1. Fracture faces of endoplasmic reticulum membranes of *Tetrahymena;* shadowing direction from below. (a) At 18°C, numerous particles distribute uniformly on the pF-faces, while the EF-faces reveal numerous depressions. (b) A large smooth area can be seen on a PF-face at 5°C. Note that particles in the particle-bearing areas are not aggregated. Magnification × 70 000.

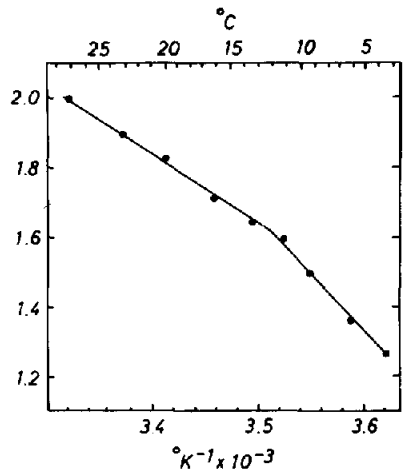

Fig.2. Arrhenius plot of the glucose 6-phosphatase associated with the smooth microsomal membranes of *Tetrahymena*. Ordinate: Logarithm of glucose 6-phosphatase activity normalized to 27°C. Note the discontinuity at ∿12°C!

3.2. *Membrane composition*

The smooth microsomal membranes of *Tetrahymena* cells grown at 18°C exhibit the same ratios of RNA/protein (0.09), phospholipid/protein (0.53) and neutral lipid/phospholipid (0.17), as well as the same SDS-gelelectrophoretic protein pattern, as those isolated from cells grown at 28°C [7]. Differences between 28°C- and 18°C-*Tetrahymena* cells can be detected only in the pattern and fatty acid composition of the phospholipids (tables 1 and 2). In comparison to 28°C-cells, the smooth microsomes of *Tetrahymena* cells grown at 18°C contain 5% more phosphatidylethanolamine and its phosphonoanalogue glycerideaminoethylphosphonate, while ceramideaminoethylphosphonate, phosphatidylinositol and lysophosphatidylcholine have decreased by about the same amount (table 1). Since we have previously shown [7] that phosphatidylethanolamine and glycerideaminoethylphosphonate contain much more unsaturated acids than the other three phospholipid species, one would expect a higher degree of unsaturation in the polar lipid and total lipid fraction, respectively, of the smooth microsomes in 18°C-cells than in 28°C-cells. Indeed, this is found as can be seen from table 2.

Table 1
Phospholipid composition of the smooth microsomal membranes of *Tetrahymena* grown at 18°C[a] and 28°C[b]

Phospholipid	Mole % of total lipid phosphorus	
	18°C-cells	28°C-cells
Phosphatidylethanolamine	31.8	30.0
Glycerideaminoethylphosphonate	26.2	23.0
Ceramideaminoethylphosphonate	12.9	14.2
Phosphatidylinositol	2.8	3.9
Lysophosphatidylcholine	2.2	4.8
Phosphatidylcholine	18.2	18.1
Monomethylaminoethylphosphonate	4.4	4.7
Cardiolipin	1.2	1.3

[a] Average of three experiments.
[b] Data from Ronai and Wunderlich (1975).

Table 2
Major fatty acid composition of the total, polar and neutral lipid fractions of the smooth microsomal membranes of *Tetrahymena* grown at 18°C[a] and 28°C[b] (% of total fatty acids)

Fatty acids	Total lipids		Polar lipids		Neutral lipids	
	18°C-cells	28°C-cells	18°C-cells	28°C-cells	18°C-cells	28°C-cells
12:0	2.5	2.8	2.9	2.9	1.3	3.6
14:0 *iso*	10.3	10.7	8.5	10.3	4.4	11.2
14:0	2.0	3.2	3.2	3.4	2.1	4.4
15:0	1.6	1.2	1.5	1.6	2.9	3.7
16:0	10.4	11.9	6.6	12.0	24.6	12.8
16:1	13.0	10.4	12.5	8.9	13.2	10.2
16:2	1.4	1.3	2.2	1.2	1.4	2.5
17:0	2.1	2.3	3.9	1.4	4.0	1.8
18:0	2.9	4.7	2.6	3.2	8.5	9.6
18:1	7.2	8.0	6.0	8.1	9.3	7.4
18:2 △ 6,9	7.7	4.2	7.7	4.7	2.3	2.8
18:2 △ 9,12	15.9	17.5	18.4	19.3	13.2	15.8
18:3	23.2	21.6	24.5	23.1	12.3	14.0
Unsaturated	68.4	63.0	71.3	65.2	51.7	52.7

[a] Average of three experiments.
[b] Data from Ronai and Wunderlich (1975).

4. Discussion

Our data show that *Tetrahymena* cells grown at 18°C have shifted the lipid clustering temperature within the endoplasmic reticulum membranes down to ∿ 12°C. Interestingly, the ratio of lipid clustering temperature to cell growth temperature remains nearly the same in *Tetrahymena* cells grown at 28°C [1]. The lowering of the lipid clustering temperature is apparently achieved by replacing the more saturated phospholipid species by more unsaturated ones. This supports the view that the poikilothermic eukaryote *Tetrahymena* is capable of adapting the fluidity of its membranes to long-time changes in environmental temperature [11,12], as it is also known in prokaryotes such as *Acholeplasma* [13,14], *Escherichia coli* [15,16] or *Bacillus* [17]. Unlike *Tetrahymena*, however, *E. coli* for instance lowers the liquid crystalline ⇌ crystalline phase transition of the membrane lipids upon lowering the cell growth temperature so that the difference between lipid phase transition temperature and growth temperature is kept almost constant [15].

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References

[1] Wunderlich, F., Ronai, A., Speth, V., Seelig, J. and Blume, A. (1975) Biochemistry, in press.
[2] Lee, A. G., Birdsall, N. J. M., Metcalfe, J. C., Toon, P. A. and Warren, G. B. (1974) Biochemistry 13, 3699–3704.
[3] Haest, C. W. M., Verkleij, A. J., DeGier, J., Scheek, R., Ververgaert, P. H. J. and Van Deenen L. L. M. (1974) Biochim. Biophys. Acta 356, 17–26.
[4] Kleemann, W. and McConnell, H. M. (1974) Biochim. Biophys. Acta 345, 220–230.
[5] Shechter, E., Letellier, L. and Gulik-Krzywicki, T. (1974) Eur J. Biochem. 49, 61–76.
[6] Eletr, S., Zakim, D. and Vessey, D. A. (1973) J. Mol. Biol. 78, 351–362.
[7] Ronai, A. and Wunderlich, F. (1975) submitted to J. Membr. Biol.
[8] Folch, J., Lees, M. and Sloane Stanley, G. H. (1957) J. Biol. Chem 226, 497–509.
[9] PintodaSilva, P. and Branton, D. (1970) J. Cell Biol. 45, 598–605.
[10] Tillack, T. W. and Marchesi, V. T. (1970) J. Cell Biol. 45, 649–653.
[11] Wunderlich, F., Speth, V., Batz, W. and Kleinig, H. (1973) Biochim. Biophys. Acta 298, 39–49.
[12] Nozawa, Y., Iida, H., Fukushima, H. Ohki, K. and Ohnishi, S., Biochim. Biophys. Acta 367, 134–147.
[13] Huang, L. and Haug, A. (1974) Biochim. Biophys. Acta 352, 361–370.
[14] Huang, L., Lorch, S. K., Smith, G. G. and Haug, A. (1974) FEBS Lett. 43, 1–5.
[15] Esfahani, M., Barnes, E. M. and Wakil, S. J. (1969) Proc. Natl. Acad. Sci. USA. 64, 1057–1064.
[16] Sinensky, M. (1974) Proc. Natl. Acad. Sci. USA. 71, 522–525.
[17] Esser, A. F. and Souza, K. A. (1974) Proc. Natl. Acad. Sci. USA. 71, 4111–4115.