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MODULATION OF MEMBRANE FLUIDITY IN A FATTY ACID AUXOTROPHE OF *TETRAHYMENA THERMOPHILA*

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Summary

A mutant of *Tetrahymena thermophila* has been isolated which requires an unsaturated fatty acid for growth. Pellicles isolated from cultures supplemented with palmitoleic, elaidic, oleic, linoleic and γ-linolenic acids show widely differing membrane fluidities, as measured by the polarisation of fluorescence technique. In contrast to the behaviour of the wild type organism, the changes in fluidity of the membrane, once induced by supplementation, are permanent. This mutant should prove extremely useful for studying structure-function relationships in the various membrane systems of *Tetrahymena*.

The regulation of membrane fluidity is known from studies on prokaryotic cells to be of importance for the control of a number of cell functions. Thus, it has been demonstrated in a number of unsaturated fatty auxotrophes of *E. coli* K12 that various biological activities are dependent on the state of the lipid bilayer [1—3]. In another well-studied system, *Acholeplasma laidlawii* B, it has been established that the physical state of the membrane lipids is correlated with both growth rates [4] and active transport [5].

Among the lower eukaryotes, fatty acid desaturase mutants of both *Saccharomyces cerevisiae* [6] and *Candida albicans* [7] have been isolated, allowing the membrane lipid composition to be manipulated. In the latter system [7] it has been shown that resistance to polyene antibiotics increases in cells supplemented with an unsaturated fatty acid.

Tetrahymena, which is also a lower eukaryote, possesses most of the

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specialised membrane systems found in higher eukaryotes, and in addition has a complex cortical organisation controlled by an intricate system of microtubules. It is thus more suited for the study of structure-function relationships than the relatively simple organisms named previously, such as yeast.

It has also been possible to modulate the lipid composition of the plasma membrane of several types of mammalian cells, for example, by culturing them in media supplemented with fatty acids [8—10]. However, these systems have proven unsatisfactory in many respects, since there is often a low uptake of the administered fatty acid [10], and the membrane fatty acid composition is usually so complex that any unequivocal interpretation of the results becomes difficult [8,9]. In one case [9], it has been reported that there is a relatively rapid return of the membrane fatty acid composition to the normal upon removing the cells from the supplemented medium.

A relatively large number of studies have appeared on the modulation of the membrane composition of *Tetrahymena*, either by supplementation of the growth medium with various additives [11—16], or by shifting the growth temperature [17—21]. In fact, *Tetrahymena* is now a much-favoured organism for the study of a number of basic membrane properties, such as membrane composition [22, 23], biosynthesis [24, 25] and turnover of phospholipid components [26, 27].

The available methods for modulating membrane fluidity in *Tetrahymena* unfortunately result in a transient change, as the organism is believed to compensate for decreasing fluidity by increasing both the level and activity of the membrane-bound fatty acid desaturases [18, 19, 21], thus regulating the relative levels of the unsaturated fatty acid [21]. One means of overcoming this regulation would be to isolate a mutant with a defect in the desaturase system, so that the organism would be dependent on the supply of externally provided unsaturated fatty acids for membrane biosynthesis. Under these circumstances, it would be expected that the membrane fluidity would change according to the nature of the fatty acid given as a supplement, unless there are compensating changes in sterol content accompanied by alterations in the polar head groups. Below, I report the isolation and partial characterization of such a mutant.

A cycloheximide-resistant strain of *T. thermophila* Chx-2/Chx-2 (cysens, IV), derived from strain B-1868, was mutagenised with *N*-methyl-*N*-nitrosoguanidine, following which the cells were mated with C*, and the short circuit conjugants cloned out, all in accordance with the procedure described by Orias and Bruns [28]. Mutagenesis, and all the following steps in the procedure (apart from the period of starvation prior to conjugation) were carried out in defined medium [29], containing a ten-fold higher concentration of biotin than that stipulated by Holz. The medium was supplemented with 10 μM each of palmitoleic acid and methyl palmitate. After growth of the clones, replica plates were made into defined medium without fatty acids, and clones selected on the basis of non-growth. These clones were then grown up in tubes, and tested more rigorously for growth in the absence and presence of palmitic and palmitoleic acids. One of the clones, RH179E1, showed an absolute requirement for growth in an unsaturated fatty acid (see Fig. 1A), and did not grow on palmitic acid alone. In the absence of an unsaturated fatty acid, the

cells died within 12—14 days at room temperature, prior to which gross morphological changes were observed, very similar to those reported by Erwin [30] in cells grown at sub-lethal temperatures. The supplemented cells show normal morphology, but are dark in appearance, perhaps because of a change in the refractive index of the membrane following the incorporation of large amounts of the supplement.

In order to establish whether the incorporation of various unsaturated fatty acids indeed led to changes in the membrane fluidity, polarisation of fluorescence measurements were carried out on isolated pellicles using the lipid probe diphenylene-hexatriene, and the procedure of Shinitzky and Inbar [31]. These results are shown in Fig. 2.

The cycloheximide-resistant strain, Chx-A2/Chx-A2 (cy sens II) (cu 329) from which the mutant was derived, was chosen as a control, and indeed, the fluidity of the freshly isolated pellicles was only slightly increased following supplementation with oleic acid, indicating that the mechanism for maintaining the correct fluidity is operating. RH179E1 pellicles isolated from cultures supplemented with palmitoleic, elaidic, oleic, linoleic and γ-linolenic acids show widely differing membrane fluidities. Using the approximation (Eqn.15

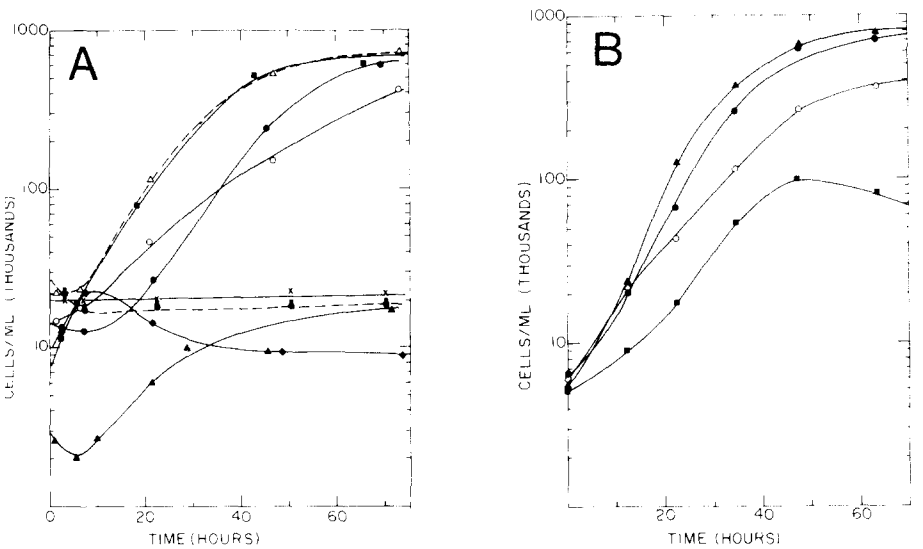

Fig. 1A. Growth curves of the mutant of *T. thermophila*, RH179E1, in defined medium [29] supplemented with various unsaturated fatty acids. Cells were taken from logarithmically growing cultures, and pre-starved for 5 days at room temperature in non-supplemented medium. Cells were then inoculated into flasks containing defined medium supplemented with 100 μg/ml of the fatty acid, 2 mg/ml fatty-acid free bovine serum albumin (Sigma) as carrier, and 50 μg/ml of α-D-tocopherol (Sigma) as antioxidant. Supplementation with palmitoleic and γ-linolenic acids was carried out with twice the concentration of albumin carrier (4 mg/ml). All cultures contained 100 μg/ml each of penicillin and streptomycin. Cell densities were measured using an electronic counter (Rasmussen, Copenhagen). Supplementation as follows: Chx-A2 cells: ○, no supplement; △, linoleic acid. RH179E1 mutant: ◆, no supplement; ×, palmitic acid; ●, palmitoleic acid; ■, oleic acid; ▲, linoleic acid and ◆, γ-linolenic acid. Fig. 1B. Growth curves of the mutant of *T. thermophila* RH179E1 in defined medium supplemented with γ-linolenic acid. Cells prestarved for 5 days in defined medium were inoculated into flasks containing defined medium with 4 mg/ml fatty-acid free bovine serum albumin as carrier and 50 μg/ml of α-D-tocopherol as antioxidant, as well as 100 μg/ml each of penicillin and streptomycin. In addition, KCl was added to three of the flasks to the following final concentrations: ▲, 25 mM; ●, 50 mM; ■, 100 mM and a fourth flask ○, no KCl (control). Cell densities were measured using an electronic counter (Rasmussen, Copenhagen).

in Ref. 32), it can be calculated that the relative viscosities of the five types of pellicle are very different: the γ-linolenic acid supplemented membranes have a fluidity some four times greater than that of the elaidic acid supplemented membranes.

The relative changes in fluidity are as expected from model studies. It has been shown that dipalmitoyl phosphatidylcholine vesicles have a lower fluidity than dioleoyl phosphatidylcholine at temperatures above the phase transition [33]. The introduction of further double bonds into the phospholipids results in the expected increase in fluidity. With the more highly unsaturated fatty acids the situation is complicated by the fact that they are highly toxic when added directly to the medium. It has been shown previously that in *Tetrahymena* γ-linolenic acid causes cell death at low doses (0.1 μM/ml) [34], although in the present study this acid was not at all toxic when sufficient carrier albumin was added. The detoxifying effect of bovine serum albumin is illustrated in Figs. 1A and 1B. In Fig. 1A, albumin carrier at a concentration of 4 mg/ml is shown to be insufficient to maintain growth of RH179E1 in a γ-linolenic acid supplemented culture. In Fig.1B, the same concentration of a different batch of albumin is shown to have resulted in good growth of the mutant, and moreover, increasing the tonicity of the medium with KCl up to 50 mM results in more rapid growth of the mutant to higher cell densities.

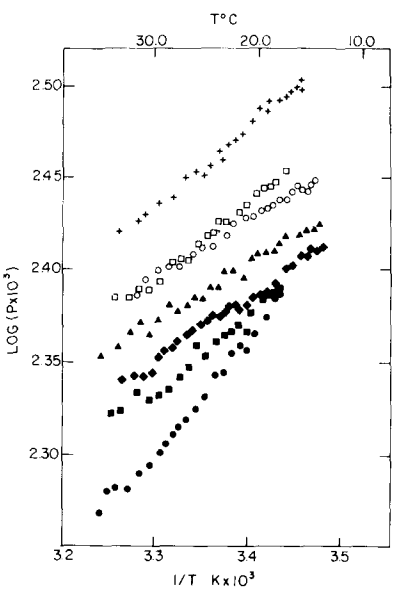

Fig. 2. The temperature-dependence of the degree of fluorescence polarisation of pellicles isolated from Chx-2/Chx-2 and RH179E1 strains of *T. thermophila*. Pellicles were isolated from logarithmically growing cells according to the procedure of Nozawa and Thompson [36]. The membrane pellet was resuspended in inorganic medium (1 mM $MgCl_2$/10 mM KCl/10 mM Tris/40 mM NaCl), and labelled in the dark with an equal volume of freshly dispersed 1,6-diphenyl-1,3,5-hexatriene in inorganic medium. Labelling was undertaken at 30.0°C, the same temperature at which the cells were cultured. After 80 min of labelling, the pellicle pellet was spun down and resuspended in inorganic medium. The degree of polarisation of fluorescence (*P*) was measured with an Elscint Microviscosimeter, Model MV-1. Chx-A2 cells: ○, no supplement; □, oleic acid; RH179E1 mutant: + elaidic acid; ■, oleic acid; ●, palmitoleic acid; ▲, linoleic acid and ◆, γ-linolenic acid.

In the wild type, the plot of fluorescence polarisation as a function of temperature shows a break at around 24°C. Similar breaks at the same temperature have been noted previously in ergosterol supplemented pellicles and microsomes of *Tetrahymena* [16], although no explanation of their cause has been forwarded. In the present series of experiments the break was only observed in freshly isolated pellicles, although the polarisation values seen at the lower temperature branch were identical in frozen preparations.

The main experimental advantages of manipulating the membrane fluidity by fatty acid supplementation of an auxotrophe are that after the initial period of change during which the fatty acid supplement becomes incorporated, the induced changes are permanent, since a regulation of fluidity by modulation of the level and activity of the desaturase is blocked in this mutant. Gas chromatographic analyses of the total cell phospholipid from cells grown with a supplement of either oleic, palmitoleic or γ-linolenic acid, together with direct assays of the enzyme activity, confirm that the lesion in this mutant is of the Δ^9 desaturase (manuscript in preparation). The permanent nature of the change in membrane fluidity is demonstrated in Fig.3, where Chx-2/Chx-2

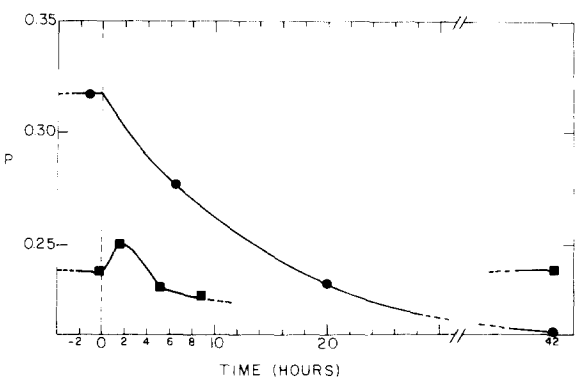

Fig. 3. Change in fluidity induced in mutant (●) and wild-type (■) *T. thermophila*. The wild type cells were grown up in defined medium alone, while the mutant cells were cultured in elaidic acid at a concentration of 100 μg/ml. Conditions of growth were otherwise as described in Fig. 1A. Cells in logarithmic growth phase were washed in inorganic medium and transferred to medium supplemented with oleic acid (100 μg/ml) at the time indicated. Pellicles were isolated at the time indicated according to the procedure of Nozawa and Thompson [36]. The pellicles were then labelled with 1,6-diphenyl-1,3,5-hexatriene as described in the text to Fig. 2, and their polarisation of fluorescence (*P*) measured at 30.0°C with an Elscint Microviscosimeter.

and RH179E1 have been subjected to the same supplementation and washing procedures, and the membrane fluidity monitored in parallel. The change in the membrane of the cycloheximide resistant strain is clearly transient, in contrast to the behaviour observed in the mutant, where after a 42-h period there is still no tendency to revert to the original membrane fluidity.

The relatively rapid compensation of the wild type membranes to the fluidity changes induced by the fatty acid supplement are in general agreement with the results of studies on the kinetics of particle rearrangement in *Tetrahymena* membrane following linoleic acid supplementation [35].

One of the immediate goals will be to confirm that the fluidity of the other membrane systems such as the endoplasmic reticulum and mitochondria can

also be modulated in a similar fashion. It is hoped that this mutant will allow detailed studied of the influence of membrane fluidity on a number of functional and structural aspects of the membrane systems in *Tetrahymena*.

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