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THERMOTROPIC CHANGES OCCUR AT FIXED TEMPERATURES IN *TETRAHYMENA* IN SPITE OF VARIATIONS IN CULTURE TEMPERATURE

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A number of experimental observations indicate that when *Tetrahymena thermophila* are grown in defined medium, a thermotropic change occurs in the membranes at about 25°C. Thus, the curve showing food vacuole formation as a function of temperature is biphasic, with a shoulder or minimum at 24–25°C. Similarly, when the maximum swimming velocity or the growth rate is measured as a function of temperature, Arrhenius plots show clear breaks at 23.2 and 23.9°C, respectively. When isolated pellicles are examined using the fluorescence of polarisation technique, a break is seen in the curves of $\ln P$ against $1/T$ at 24.6°C, demonstrating that a phase change occurs at this temperature. Furthermore, this same phase change was demonstrated in the pellicles of a fatty-acid-requiring mutant of *T. thermophila*, RH179E1, at 25.2°C. A review of the literature revealed that in cells cultured in peptone media, there is evidence of thermotropic changes in many *Tetrahymena* membranes around 25°C, but that in addition, changes also occur around 16°C. The experiments on the temperature variation of swimming and growth were therefore repeated in peptone media, and it was confirmed that, in contrast to defined medium, two break points are always seen in Arrhenius plots. In particular, it was noted that these break points are practically invariable, having values of 25.8 and 15.6°C, a fact that is of considerable theoretical interest. The experiments described here and in the literature demonstrate that a wide range of biological functions are apparently correlated with thermotropic changes in the membranes occurring at 25°C and 16°C.

Introduction

2 years ago, a mutant of *Tetrahymena thermophila* was isolated which requires an unsaturated fatty acid for growth [1]. The membrane fluidity of this mutant can be modulated over a wide range, making it a potentially useful cell for studying various membrane associated processes, such as conjugation [2] and food vacuole formation [3].

Preliminary studies in this laboratory had shown that food vacuole formation in the mutant at 30°C was severely impaired in comparison with the wild type. As very little was known about the factors controlling food vacuole formation in *T. thermophila*, it was decided to carry out certain control experiments in order to elucidate the malfunctioning of the mutant.

These experiments, described in detail below, revealed that the curve depicting food vacuole formation as a function of temperature is biphasic, with a shoulder or minimum at 24–25°C. Further experiments confirmed the existence of a single thermotropic discontinuity at 24°C in cells cultured in defined medium. In contrast, it appears

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that when cells are grown in peptone media, two apparently invariable thermotropic discontinuities are seen. The significance of these findings will be discussed.

Materials and Methods

Cultures of *T. thermophila* Chx-2/Chx-2 (cysens, IV) (hereafter referred to as *T. thermophila* BIV) were grown in defined medium [4] containing a concentration of biotin 10-fold higher than that stipulated by Holz. Supplementation of this medium with fatty acids was carried out as described previously [1]. 50 ml cultures were grown in 250 ml Erlenmeyer flasks, without agitation, at 30°C. In subsequent experiments, a medium consisting of 1% bacterial peptone (L37 Oxoid) and 0.1% yeast extract (L21 Oxoid), supplemented with 36 μM $FeCl_3$ was used. For experiments with *Tetrahymena pyriformis*, the GL strain was used. The *T.* thermophila mutant RH179E1 has been described previously [1].

The swimming velocity was measured on a stage, the temperature of which was measured by a Mettler TM1 thermistor and was maintained to within 0.05°C by circulation of water from a thermostatically controlled bath.

A single drop of cells was placed between two cover slips on the cleaned glass surface of the stage, and the cells were covered with a third cover slip. The preparation was then covered with a thin (0.4 mm) piece of brass sheet pierced with a hole 1.04 mm in diameter. Exactly 6 min after having placed the sample on the stage, the times taken for single cells to traverse the full diameter of the hole were noted to within 0.1 s in quick succession for a period of between 6 and 8 min. Between 15 and 30 measurements were taken in this period of time. After removing the cells and coverslips, the stage was scrupulously cleaned with saline, distilled water and finally 96% ethanol before commencement of the next measurement. The method of transit frequency, described by Tawada and Oosawa [5] for *Paramecium* was also tried, but was found to suffer from errors due to sampling and to the difficulty of making layers of well-defined thickness.

For measurements of the rate of food vacuole formation and growth, a special thermostat device was constructed as follows. A solid brass block, 6×6 cm in cross-section and 40 cm long, was machined with a 6 mm diameter transverse hole at one end, to accept a thermostat-controlled heating finger. At the other end a 25 mm hole was drilled through and connections made to accept coolant from a Hetofrig thermostat (Heto, Birkerod, Denmark). On the upper face of the block, a small vertical hole was made 3 cm from the heating element to accept a thermistor connected to the thermostat which controlled the heating element (70–100 W was the ideal range). Further down the block, at 3-cm intervals, holes were drilled to accept eight test tubes. The cool end of the block was maintained at −16°C, and the hot end at 30–45°C, depending on the temperature range required. After 3 h of equilibration, the temperature in each tube, when filled with 2–3 ml of fluid, was constant to within 0.1°C over an indefinite period.

For measurement of the rate of food vacuole formation, 3 ml of an early log phase culture of *Tetrahymena* were placed in each tube, and after 1 h equilibration [6] Indian ink (Rotring Art. No. 591017) was added to give a final dilution of about 1500-fold [7]. After 10 min, samples were taken for counting after fixation in 2% formaldehyde. The temperature in each tube was measured with a Mettler TM1 thermistor to within 0.05°C. Experiments showed that within the range 1000–3000-fold dilution of Indian ink, there was no significant variation in the number of food vacuoles formed.

Determination of the generation time was carried out in 3 ml cultures set up in the eight tubes of the brass block. 200-μl samples were taken at noted times and fixed in 200 μl 4% formaldehyde, and after dilution with 1.6 ml 0.9% NaCl, the 90° scatter of the samples at 350 nm was measured in a 10 mm cuvette in an Aminco Bowman spectrophotofluorimeter. The relationship between the scatter at 350 nm (S_{350}) and the absolute cell count showed that S_{350} was proportional to the cell density up to 6500 cells/ml in final dilution. The generation time, T_g, was determined from plots of S_{350} against time.

The preparation of pellicles and fluorescence polarisation measurements on these membranes after labelling with diphenylenehexatriene (DPH)

were carried out as described previously [1,8].

Supplementation of cultures, both wild-type and the mutant RH179E1, were carried out as described previously [1].

The slopes in the Arrhenius plots were calculated by the method of least-squares, and the intercepts from these statistically calculated slopes as described in Documenta Geigy, 6th edition.

Results

Cultures of *T. thermophila* BIV were grown in defined medium at 30°C, and their capacity to form food vacuoles measured as described under Methods. The results of two typical experiments are shown in Fig. 1, from which it is clear that the rate of formation of food vacuoles does not decrease regularly on decreasing the temperature, but in fact is biphasic, with a point of inflection at 25°C. Below 12°C, no food vacuoles are formed in 98% of the cells.

Experiments were also carried out to study the effect of supplementation of the *T. thermophila* BIV cultures with oleic acid (Fig. 2). The most obvious effect of the supplementation is to reduce the rate of food vacuole formation to under a half of that seen in defined medium alone. The greatest reduction is seen at temperatures above 25°C, so that after 18 h growth the biphasic character of the vacuole formation curve is accentuated. After 2 days in the oleic acid-supplemented medium, the food vacuole formation above 25°C is at about one-third of its original level, so that there are now two peaks, with a clear minimum at 24°C. The biphasic nature of the curves shown in Fig. 2 suggests that above and below 25°C food vacuole formation takes place by two different processes with different temperature dependencies, and that the process taking place above 25°C can be significantly suppressed by supplementation with oleic acid. The simplest hypothesis to explain this selective suppression would be that massive incorporation of an unsaturated fatty acid into the endocytotic membranes renders them too fluid to function effectively above 25°C. Unfortunately, this hypothesis would be difficult to verify experimentally, as a technique for the isolation of food vacuole membranes in sufficient quantities to carry out lipid analyses and to make estimates of membrane fluidity does not as yet exist.

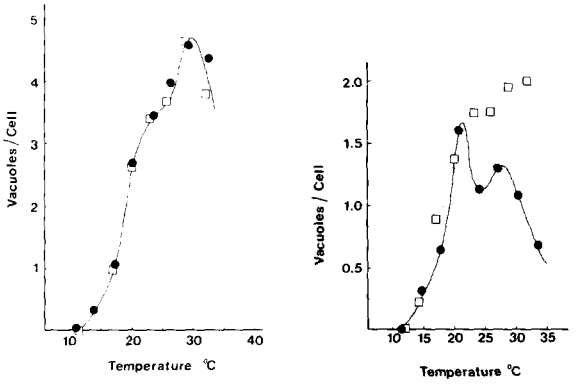

Fig. 1. Rate of food vacuole formation in *T. thermophila* BIV cultured in defined medium at 30°C. The ordinate indicates the number of vacuoles formed during a 10-min period after the cells have been equilibrated at the given temperature for 1 h. □ and ● are points obtained using two different cultures set up under identical conditions.

Fig. 2. Effect on food vacuole formation of supplementation of the growth medium with oleic acid. *T. thermophila* BIV was cultured at 30°C in defined medium supplemented with 50 μg/ml oleic acid for 18 h (□) and 2 days (●). See Fig. 1 for experimental details.

An earlier study by Tawada and Oosawa [5] on the swimming velocity of *Paramecium caudatum* showed that the maximum velocity was always seen at the temperature at which the cells were cultured, the swimming rate decreasing sharply at either higher or lower temperatures. The ion pumps and channels critical to the regulation of swimming activity in *Paramecium* are located in the cell-surface membrane [9,10], and the electrophysiological properties of *Tetrahymena* are known to parallel closely those of *Paramecium* [11]. Since *Tetrahymena* is capable of regulating its membrane composition in response to changes in culture temperature [12–14], it was of interest to see whether there were any parallel changes in the swimming velocity, as observed with *Paramecium*.

A typical result for a 30°C culture of *T. thermophila* BIV is shown in Fig. 3. Unlike *Paramecium*, the swimming velocity of *Tetrahymena* is not maximal at the culture temperature (see also Fig. 8), but the Arrhenius plot has a sharp break at 24.7°C. The activation energies are 41.2 and 83.4 kJ/mol above and below the break point. In a quadruplicate experiment for cells cultured at 30.0°C in

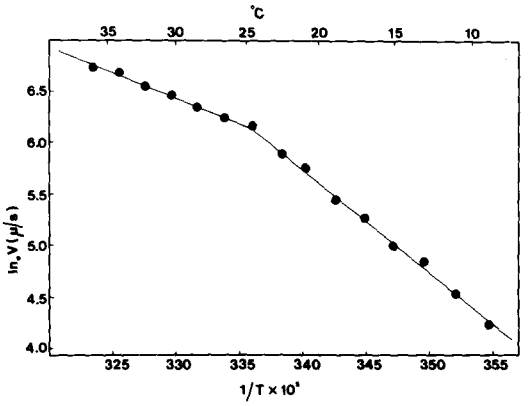

Fig. 3. Swimming velocity of *T. thermophila* BIV cultured in defined medium at 29.8°C. The velocity was measured 6 min after transfer of a drop of the culture to a thermostatically controlled microscope stage. Each point represents the mean of at least 15 measurements over a 6–8-min period. The slopes and intersection point (24.7°C) are statistically calculated.

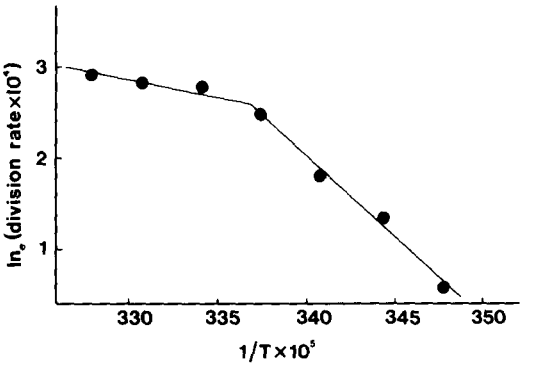

Fig. 4. Growth rate of *T. thermophila* BIV cultured in defined medium at various temperatures. Each point is the mean of duplicate determinations of the division rate, expressed as cell divisions per min.

defined medium, the mean break point was 23.2 ± 1.4°C, and the activation energies above and below the break point were 39.7 ± 5.9 and 104 ± 15 kJ/mol, respectively.

It now seemed clear that some membrane change occurs at about 24°C in cultures of *T. thermophila* BIV grown in defined medium. Since it has been known for a long time that the generation time of *T. pyriformis* cultures is very sensitive to temperature [15–17], it was decided to examine the growth rate of *T. thermophila* BIV in defined medium. The results, presented in Fig. 4, show a clear break in the Arrhenius plot at 23.9°C (as compared with 23.2°C for the swimming velocity plots and 24°C for the food vacuole formation curves). The activation energies above and below the break point are 33.9 and 148 kJ/mol, respectively.

The experiments presented in Figs. 1–4 all show a clear change in the rates of endocytosis, swimming and growth at around 24°C. Furthermore, other fluorescence polarisation studies on isolated pellicles of *Tetrahymena* have indicated breaks around 22–25°C in plots of fluorescence polarisation against temperature [1,18]. These breaks have also been reported using the same technique in lipid extracts obtained from *Tetrahymena* microsomes [19]. In order to confirm and extend these findings, fluorescence polarisation measurements have been carried out on pellicles of both the wild-type *T. thermophila* BIV and the mutant RH179E1 grown in defined medium supplemented with various fatty acids. Fig. 5 shows the results of four such experiments. All the curves show departures from linearity around 25°C, confirming the existence of a phase change at this temperature in spite of the differences in 'microviscosity'. The temperature at which this phase change occurs is in good agreement with the temperatures at which changes in swimming velocity, etc. occur. For the wild-type *T. thermophila* BIV, the mean break temperature for three experiments was 24.6 ± 2.2°C, and for ten experiments with the mutant grown with various unsaturated fatty acid supplements the break temperature was 25.2 ± 1.3°C. Lipid analyses on control and supplemented cells have been carried out, and these results will be published later; in each case, the supplement caused an increase in the relative mole fraction of the administered fatty acid.

A review of the literature on the response of *Tetrahymena* to temperature shows that nearly all the experiments have been carried out in complex (undefined) media, e.g., proteose or bacterial peptone with a supplement of yeast or liver extracts. In order to establish whether differences in culture medium could influence the temperature response, many of the experiments described above were repeated on cells grown in bacterial peptone supplemented with yeast extract.

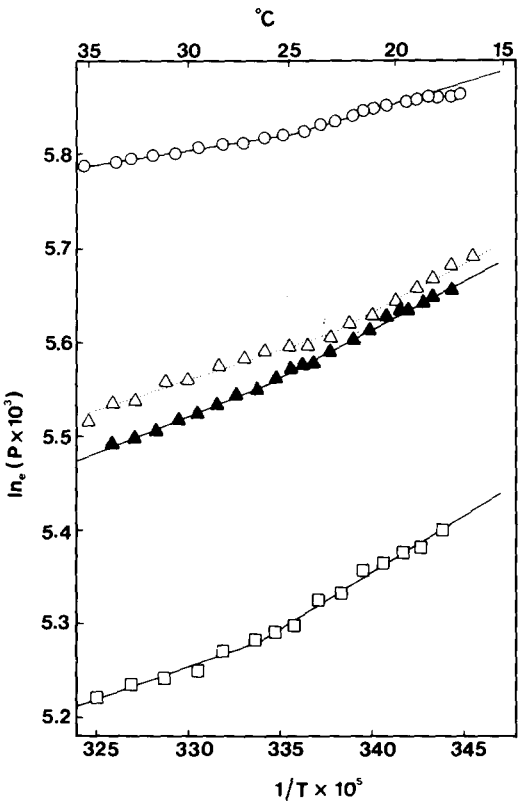

Fig. 5. Polarisation of fluorescence (P) measurements on pellicles isolated from *T. thermophila* BIV and a fatty acid auxotrophe RH179E1. Pellicles were isolated and labelled with the fluorescent probe DPH as described in Materials and Methods. ▲, wild-type; △, wild-type grown in defined medium supplemented with oleic acid; ○, auxotroph grown in defined medium supplemented with elaidic acid; □, auxotroph grown in defined medium supplemented with oleic acid.

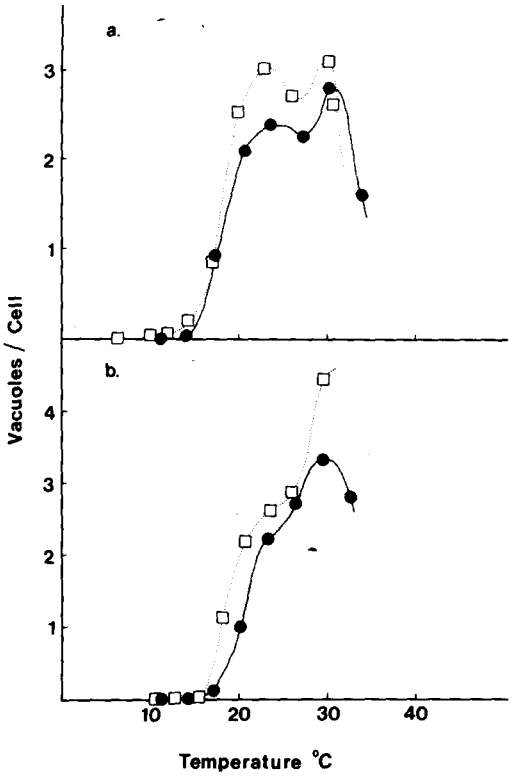

Fig. 6. (a) Rate of food vacuole formation as a function of temperature for the amicronucleate *T. pyriformis* GL, cultured in bacterial peptone/yeast extract at 28°C. □ and ● are points obtained using two different cultures set up under identical conditions. (b) Corresponding curves for *T. thermophila* BIV cultured in the same medium at 30°C. □ and ● are points obtained using two different cultures. In both (a) and (b) the ordinate indicates the number of vacuoles formed during a 10-min period after the cells had been equilibrated at the noted temperature for 1 h.

Fig. 6a shows the rate of food vacuole formation as a function of temperature for a 28°C culture of the amicronucleate strain *T. pyriformis* GL. Fig. 6b shows the corresponding curves for *T. thermophila* BIV cultured at 30°C. As before (Fig. 1), the curves are biphasic with shoulders or minima close to 25°C. However, comparison of Figs. 1 and 6b shows that in bacterial peptone, food vacuole formation has ceased by 16°C, whereas in defined medium at the same temperature there is still significant formation of vacuoles (Fig. 1).

Fig. 7 shows the combined results from three of five experiments on the rate of reproduction of *T. thermophila* BIV in bacterial peptone as a function of growth temperature. When compared with Fig. 4, it appears that there are two break points on the curve, one at 23.6 ± 0.9°C (as compared to 23.9°C in defined medium, Fig. 4), and in addition a sharp break at 15.6 ± 0.4°C. The activation energies for the three parts of the Arrhenius plot are 23.1, 48.0 and 250 kJ/mol. In defined medium, on the other hand, the activation energies (Fig. 4) are 33.9 and 148 kJ/mol, values almost exactly intermediate with the values obtained in bacterial peptone. It is thus quite clear that the growth characteristics and membrane properties of *T. thermophila* BIV are different when the cells are cultured in peptone and defined media.

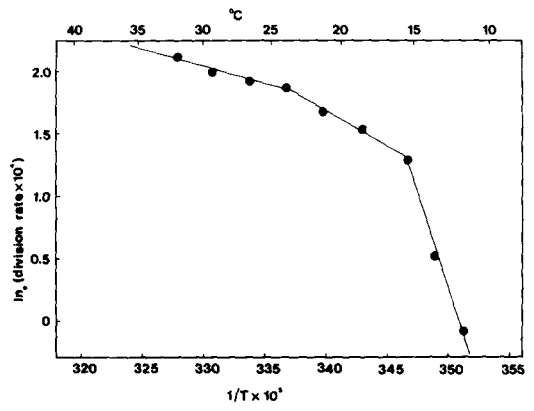

Fig. 7. Growth rate of *T. Thermophila* BIV cultured in bacterial peptone/yeast extract at various temperatures. Apart from the lowest temperature point, which is the mean value of duplicate determinations of the growth rate (expressed as cell divisions/min), each point is the mean of triplicate determination. The slopes and intersection points (23.6 and 15.6°C) are statistically calculated.

Fig. 8 illustrates the rate of swimming of *T. thermophila* BIV as a function of temperature. Cells were cultured in the same batch of medium at various temperatures between 13°C and 38°C for a length of time sufficient for the cells to go through at least six generations. In addition, the cells were transferred at least once, the concentration of cells after the final transfer being chosen such that the culture used for the experiments contained cells at a low density $((10–20)\cdot 10^3$ cells/ml). The motility of the cells was measured as described in Materials and Methods, and the results are presented as Arrhenius plots of ln velocity against the reciprocal of the temperature. In Fig. 8, for the sake of clarity, only four representative curves are shown, the rest of the data using this same batch of medium are presented in Table I.

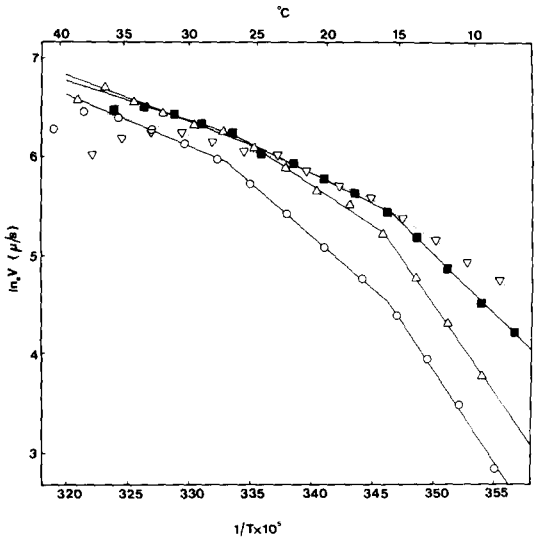

Fig. 8. Swimming velocity of *T. thermophila* BIV cultured in bacterial peptone/yeast extract at various temperatures; ▽, 13.90°C; ■, 23.35°C; △, 33.35°C, and ○, 38.05°C. Further experimental details are given in the text to Fig. 3. The upper and lower break-point temperatures for each experiment are given in Table I, together with data on several other experiments carried out with cells cultured at intervening culture temperatures.

Two important facts should be noted; firstly, the break temperatures differ by only 3°C over the whole temperature range. From the data presented in Fig. 8 and two similar experiments carried out on cells cultured at 30°C in different batches of medium, mean values for the upper and lower break temperatures were calculated as 25.83 ± 1.41°C and 15.60 ± 1.07°C, respectively ($n = 12$). The variation of the upper and lower break temperatures with culture temperature was examined statistically. Using Bartlett's test, it was shown that the sample variances were not significantly different, and thereafter it was shown that the break-point temperatures did not vary significantly with the culture temperature. This is in contrast with results reported by Dickens and Thompson [19] for lipid extracts of *Tetrahymena* cultured at different temperatures. In these studies, the break points in the Arrhenius plots shifted 7–8°C on lowering the culture temperature from 39.5°C to 15°C. The second point to note is that below the upper break temperature the activation energies (E_a) change in a systematic manner as the culture temperature is changed (Fig. 9). The statistical validity of the two upper curves shown in Fig. 9 was tested and found to be significant at the 0.1% level, in contrast to the plot of E_a against culture temperature for the activation energies measured above 25.8°C (lowest curve). Here, E_a does not change significantly with culture temperature, the mean value being 32.75 ± 7.80 kJ/mol. This value is typical for membranes in a fluid state [20] and is in excellent agreement with that calculated from theoretical considerations [21].

TABLE I

BREAK-POINT TEMPERATURES AND ACTIVATION ENERGIES CALCULATED FROM ARRHENIUS PLOTS OF SWIMMING VELOCITY OF *T. THERMOPHILA*

The swimming velocity was determined as described in the text to Fig. 8. The upper and lower break points do not vary significantly with culture temperature, as demonstrated by Bartlett's test.

Culture temp. (°C)	Break-point temp. (°C)		Activation energies (kJ/mol)		
	Lower	Upper			
13.90	16.71	23.31	25.63	46.22	67.21
18.30	14.46	23.98	19.51	52.86	94.25
23.35	15.53	26.77	32.58	51.08	101.4
28.30 ($n = 2$)	13.91	25.63	33.35	65.48	128.9
33.35	16.11	25.30	39.51	67.80	148.2
37.80 ($n = 3$)	16.11	26.59	41.05	81.46	167.7
38.05	14.94	26.03	37.63	78.36	159.8

Thus, as the cell culture temperature is increased, the activation energy also increases, indicating that the membrane is more fluid after adaptation to growth at low temperatures. This is in good agreement with what has been shown previously in various *Tetrahymena* membranes [22–24]. Of particular interest is the observation that the activation energy at each culture temperature, when read off from the Arrhenius plots, is held within the rather close limits of 46.1 ± 11.7 kJ/mol. Thus, as suggested previously [25], the membrane synthesis of *Tetrahymena* is tightly controlled to give membranes of very similar properties at different growth temperatures.

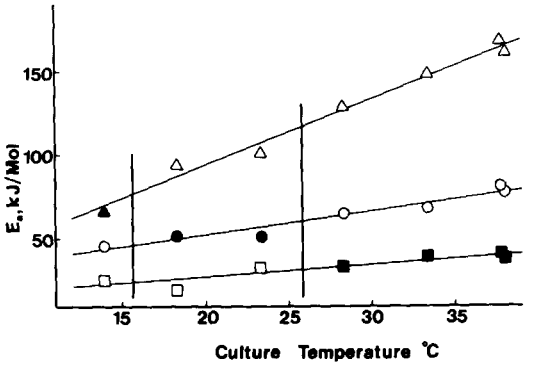

Fig. 9. Variation of activation energies with culture temperature. The activation energies, taken from Table I, are calculated from the slopes of Arrhenius plots of swimming velocity of *T. thermophila* BIV cultured in bacterial peptone/yeast extract, some of which are shown in Fig. 8. □, activation energies calculated from the slopes of the lines above the upper break-point temperature of 25.8°C. △, activation energies calculated from the slopes below the lower break-point temperature of 15.6°C. ○, activation energies calculated from the slope of the line extending between the two break-point temperatures. ■, ▲ and ● are the values of the activation energy at the culture temperature, which are seen to decrease with increasing culture temperature. The two vertical lines mark the temperatures at which breaks occur in the Arrhenius plots of swimming velocity.

Discussion

In this paper, data have been presented to show that, irrespective of the culture medium, food vacuole formation in *Tetrahymena*, as well as the rates of growth and swimming, all show a marked temperature dependence with an abrupt change occurring around 25°C. Furthermore, when the cells are cultured in a bacterial peptone/yeast extract medium, an additional change is observed around 16°C. Studies using the fluorescence polarisation technique have demonstrated that in pellicles isolated from cells grown in defined medium, a change in membrane fluidity occurs at 25°C.

The difference in behaviour noted between cells cultured in the two different media is not only limited to the presence or absence of the 16°C break point in Arrhenius plots. For both the swimming and the growth-rate experiments (Figs. 3 and 9, 4 and 7), the activation energies in defined

TABLE II

SURVEY OF LITERATURE RECORDING THERMOTROPIC EVENTS IN *TETRAHYMENA*

Cell strain	Culture medium and growth temp (°C)	Material studied	Experimental method	Break temp. [a] (°C)		Ref.
T. pyriformis WH-14	proteose peptone, 25	pellicle lipid vesicles	ESR	17	27	22
T. pyriformis GL	Proteose peptone, 28	microsomes	NMR, ESR, glucose-6-phosphatase assay	17	[b]	53
T. pyriformis NT-1	proteose peptone, 39.5	microsomes	stearoyl CoA and palmitoyl CoA desaturase assay	15/15,	26/30	58
T. pyriformis NT-1	proteose peptone, 39.5	pellicles	adenyl cyclase assay	15	28	59
T. pyriformis NT-1	proteose peptone, 39	pellicles and microsomes	polarisation of fluorescence	– [c]	22–25	18
T. pyriformis NT-1	proteose peptone, 39	pellicles	ATPase assay		27	60
T. pyriformis NT-1	proteose peptone, 39.5	smooth microsomes	glucose-6-phosphatase assay	–	25(23/28) [d]	32
T. pyriformis NT-1	proteose peptone, 39.5	smooth microsomes	palmitoyl CoA desaturase assay	–	31	32
T. pyriformis NT-1	proteose peptone, 39/15	microsomal lipids	polarisation of fluorescence	15/8 [e]	28/20	19
T. pyriformis GL	proteose peptone, 38-18	whole cells	growth rate in capillaries		22	15
T. pyriformis HS	proteose peptone, 38-18	whole cells	growth rate in capillaries		23.5	15
T. pyriformis GL	proteose peptone, 33-10	whole cells	growth rate of single clones	16.7	23	16
T. pyriformis, syng 1	aerobacter-cerophyll 26-15	whole cells	testing for serotype with specific antibodies	15	25	47
T. pyriformis, syng 1	peptone and aerobacter-cerophyl 28-15	whole cells	testing for serotype with specific antibodies	15	22-28	48
T. pyriformis GL	proteose peptone, 23/18	whole cells	transport and synthesis of RNA	15/13 [f]		61
T. pyriformis GL	bacterial peptone, 28	whole cells	fool vacuole formation	[g]	26–27	p.s [h]
T. thermophila	bacterial peptone, 30	whole cells	food vacuole formation		25	p.s.
T. thermophila	bacterial peptone, 30	whole cells	swimming velocity	15.6	25.8	p.s.
T. thermophila	bacterial peptone, 30	whole cells	growth rate	15.6	23.6	p.s.
T. thermophila	defined medium, 30	pellicles	polarisation of fluorescence	–	24.6	p.s.
T. thermophila	defined medium, 30	whole cells	food vacuole formation	–	24–25	p.s.
T. thermophila	defind medium, 30	whole cells	growth rate	–	23.9	p.s.
T. thermophila	defined medium, 30	whole cells	swimming velocity	–	23.2	p.s.

medium are seen to be intermediate to the values obtained in bacterial peptone and it is clear that the properties of the membranes in the cells cultured in the two different media are different. As will be discussed later, temperature adaptation in *Tetrahymena* is a complex process which is not complete until after a lag phase of several hours. During the course of accumulating the growth data shown in Figs. 4 and 7, it was noted that after transfer of cells from 30°C to some other temperature under 25°C, only a very brief lag period was seen in defined medium. This was in marked contrast to the behaviour in peptone medium, where the lag period lasted as long as 20 h after transfer of cells to 10°C or lower. The absence of a lag phase and the different growth rates show that both the immediate and the long-term responses to a temperature shift are quite different in cells grown and maintained in defined medium as compared to peptone medium. The experiments shown in Figs. 7 and 8 were both carried out with cells fully adapted to various culture temperatures, i.e., grown at that temperature for a sufficient length of time for the cells to have divided at least six times, and demonstrate that 16°C and 25°C represent fixed break points which are always maintained by the organism in the face of large temperature shifts.

Break points have previously been noted in the case of *Tetrahymena*, as seen from a survey of some of the literature which has been assembled in Table II. This table is divided into three sections, the first two of which review the literature of experiments carried out on *Tetrahymena* cultured in peptone or peptone-supplemented media, while the last section summarises the results obtained in the present paper using cells cultured in defined medium.

Examination of the table reveals that the differences in the break-point temperatures recorded for the various strains of *Tetrahymena* are small, even when the cells are cultured under different conditions. Neither does it appear to matter much which membrane system is examined nor which experimental technique is used, in spite of the criticism which has been levelled against some of the methods utilised. In particular, there has been considerable debate in the literature as to the validity of using the fluorescence polarisation method for quantitative estimation of membrane microviscosity [26–28]. However, for qualitative comparisons between membranes or lipid vesicles of low protein content and for registration of transition temperatures, the method is valid [29]. In membranes or lipid vesicles of high protein content, on the other hand, the polarisation values increase, and this in now believed to be due to interaction of the probe molecule with the intrinsic proteins [30]. Electron spin resonance (ESR) probes can also react with membrane proteins in a similar way [30]. These probe-protein interactions explain why the native membranes studied in *Tetrahymena* appear to be less fluid than vesicles prepared from lipid extracts of these same membranes [31,32]. At the same time it should be noted that the 'boundary lipid' previously invoked to explain the apparently higher fluidity of lipid extracts has now been shown to be an artefact of the ESR technique [33,34].

Several of the experiments referred to in Table II have involved measurement of the activity of a membrane-bound enzyme as a function of temperature, and a presentation of the results in the form of Arrhenius plots. In spite of some criticism of the assumption that abrupt changes in the slopes of Arrhenius plots can be attributed to lipid phase changes [35], in a number of cases a correlation has been found between changes in enzymatic

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[a] All temperatures quoted are approximate values, unless given with decimal place.

[b] Where no break temperature is indicated, experiments were not conducted within the appropriate temperature range.

[c] – signifies the absence of a break in the 13–17°C range.

[d] Values in brackets are for two different smooth microsome fractions; value outside brackets is for unfractionated smooth microsomes.

[e] Break temperatures for the 39°/15°C cultures, respectively.

[f] The statistical precision of these break points is low. Temperatures are for the 23°/18°C cultures, respectively.

[g] It is not possible to localise the lower break temperature from this type of experiment.

[h] Present study.

activity and alterations of the physical state of the membrane [36,37]. From Table II it can be seen that the break-point temperatures obtained from Arrhenius plots certainly correlate well with the polarisation and ESR data. Not all reports in the literature show the existence of break points. In a recent paper [38], Ramesha and Thompson have looked at the thermotropic behaviour of cilia, and, in contrast to microsomes, no break points were observed in plots of fluorescence polarisation against temperature. In these experiments total lipids, ciliary phospholipids and membrane vesicles all gave practically linear plots. However, preliminary findings in our laboratory, using ESR and ^{31}P-NMR on cilia preparations indicate that in intact ciliary membranes, thermotropic changes do occur.

While a large number of studies have been made demonstrating thermotropic events in isolated membranes, until now relatively few studies have been made on viable cells. The advantages of studying membrane phenomena in intact, viable cells are numerous. When separating membranes or lipids, the lipid components are subjected to lipolytic action [39], and as a result there are usually significant amounts of lysolipids, which are known to affect membrane properties [40,41]. Also, lipid extracts usually differ in their physicochemical properties from the membranes from which they are derived (see, for example, Refs 22, 31, 32). Furthermore, the phospholipid fractions show higher break-point temperatures than total lipid samples in fluorescence polarisation experiments [42]. If *Tetrahymena*, in common with the cells of many other organisms, shows membrane assymetry [43] and perhaps a lack of bilayer coupling [44–46], it is readily understood how any disturbance of the membrane integrity could change the physicochemical properties. In summary, it seems reasonable to suppose that experiments using intact, viable cells come closer to describing the true thermotropic behaviour of the external cell membranes than do those involving isolated membranes or lipid extracts.

Of the data presented in the second section of Table II, the experiments of Nanney [47] and Juergensmeyer [48] deserve particular comment. In 1958, Loefer et al. [49] noted that cultures of some strains of *Tetrahymena* had a different antigenic specificity according to the temperature at which they were cultured. Subsequent studies [47,48] demonstrated the presence of four mutually exclusive serotypes, L, H, T and I. The L serotype is expressed at 15°C or below, the H serotype at 22–28°C, and a T antigen above 36°C. The I antigens, which transform frequently amongst themselves, are induced by treatment of cells at room temperature with an anti-H antiserum. Extensive crosses have been carried out in order to establish the genetics of the system [48]. It appears that it is the environment which determines the serotype, and that the temperature at which the cells are placed determines the speed of the transformation. Thus, L cells (incubated at 15°C), will transform almost immediately to H at 28°C, but after transfer to 22°C, many cells will retain L, and if placed at 19°C, most cells will be L even after a number of generations. On the other hand, Nanney showed [47] that the serotypes of exconjugant clones produced in crosses between H and L cultures were not systematically different.

A simple explanation for the transformation of serotypes is that they are a further consequence of the same membrane changes which are reflected in all the other thermotropic phenomena listed in Table II. These changes in membrane state could affect the conformation (antigenicity) of the immobilisation antigens, or, as suggested by Borochov and Shinitzky [50], there may be vertical displacements of the proteins in the membrane. The temperatures at which the L and H serotypes express themselves correspond well with the break temperatures shown in Table II. The T serotype occurs at the temperature of maximum swimming velocity seen in the Arrhenius plots of cells grown above 30°C (Fig. 8).

The second section of Table II also contains a summary of the results of experiments on the swimming rate as a function of temperature. One interesting result is that above the upper break temperature, 25.8°C, the activation energy is practically invariant with culture temperature, the mean value being 32.8 kJ/mol. In a theoretical treatment of phospholipid lateral diffusion in liquid crystalline membrane bilayers, Tinker [21] calculated a value for the activation energy for viscous flow, assuming a diffusion coefficient of $D = 1 \cdot 10^{-8}$ cm$^2 \cdot$ s^{-1}. He found that the energy barrier to

lateral motion was 30.2 kJ/mol, a value very close to the experimentally determined mean value given above. The identity of these two values suggests that above 25.8°C, the upper break temperature, *Tetrahymena* membranes are in the liquid-crystalline state.

Comparison of Fig. 3, which shows the swimming velocity as a function of temperature, with Fig. 5, which shows that the fluidity of the outer membrane system decreases with temperature, suggests that the swimming velocity is a function of membrane fluidity. Goto et al. [52] have, in fact, using ESR spectroscopy to measure membrane fluidity, directly verified this correlation. As discussed previously, the regulation of ciliary beat is associated with ion fluxes through channels in the surface membrane [10]. If one assumes a strong coupling between the processes of lipid flow and the activation of the channel, it can be predicted that the maximum rate of channel functioning will be proportional to the membrane fluidity up to a certain maximum value [51], in accordance with the experimental evidence above. Examination of Fig. 8 reveals that at any one particular temperature below 25°C, the swimming velocity increases as the cell culture temperature is decreased. Since the swimming velocity is related to the membrane fluidity, this suggests that the membrane fluidity increases with decreasing culture temperature, in agreement with previous findings [22]. However, at the culture temperature itself, the fluidity appears to decrease with temperature.

The question then arises as to the nature of the thermotropic events occurring in the membranes at the two fixed temperatures, 16°C and 25°C. The simplest explanation is that they represent the upper and lower limits of a broad phase transition. This suggestion was earlier considered by Wunderlich [53], but rejected on the basis of lack of support from differential scanning calorimetry. Dickens and Thompson [19] are inclined to interpret the break points seen in microsomal lipid vesicles as representing temperatures at which some molecular species go from a disordered to an ordered state on cooling, and suggest that only small structural changes take place at these temperatures. The very small changes in activation energy which are seen in their experiments also indicate relatively minor reorganisations in the vesicle membrane. In the experiments reported in this paper (Fig. 5), the changes in slope in the fluorescence polarisation curves are also small. By contrast, the changes in the activation energy which occur at the break points of the Arrhenius curve are large (Table I), and suggest that at these temperatures considerable changes occur in at least some locations within the membrane. However, at present we do not know the nature of the events occurring at and between the two break-point temperatures, and have therefore initiated work to fill this gap in our knowledge of *Tetrahymena* membranes.

As discussed previously, cells grown in defined medium, in contrast to those grown in peptone, do not have a lag period following a temperature shift. This, together with the other differences noted for cells grown in the two types of medium, suggests that in defined medium *Tetrahymena* adjust their membrane fluidity in a different way, and have an impaired ability to synthesise the necessary regulatory components possibly because the defined medium lacks some essential precursor or factor. It might well be that a comparison of the phospholipid composition of *Tetrahymena* thermophilia cultured at various temperatures in the two media would provide an indication of which structural groups are responsible for the observed differences.

That the break points always occur at approximately the same temperatures in *Tetrahymena* is intriguing. In more primitive unicellular organisms, such as *Escherichia coli* and algae, shifts in the growth temperature are usually followed by changes in the fatty acyl composition, and these in turn are reflected in shifts of the break points when some physiological function is monitored as a function of temperature [54–56]. That such shifts in break-point temperatures are not observed in *Tetrahymena*, in spite of large differences in the fatty acyl composition of the membranes, suggests that some compensatory mechanism is operating. One possibility is that the system responsible for regulating the unsaturated fatty acid content, and the headgroup incorporation system, are coupled with each other in such a way that a change in acyl chain fluidity induces alterations in the headgroup composition. Such a compensation could prevent a shifting of the break points in response to a change

in fatty acyl composition, and would also stabilise the membranes by ensuring that the cross-sectional areas of the hydrophobic and head group regions would remain approximately the same [57]. This mechanism could maintain the break points at fixed temperatures in spite of large individual differences in composition, fluidity and function between the various membranes.

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