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LARGE-SCALE SELECTION SYNCHRONY OF *TETRAHYMENA THERMOPHILA*

ROBIN J. HILL, T. KROFT, M. ZUKER AND IAN C. P. SMITH
Division of Biological Sciences, National Research Council, 100 Sussex Drive, Ottawa, K1A 0R6, Canada

SUMMARY

A method is described, based on the phagocytosis of colloidal ferrite particles, which gives highly synchronous populations of *Tetrahymena thermophila*. To ensure a successful synchrony, the cell culture doubling time, the limits of the phagocytic period and the distribution of cell stages must first be determined. Once these parameters are known, synchrony can be achieved under a variety of growth conditions and with cultures ranging in volume from a few millilitres to 12 litres or more.

The main advantages of the method are that the apparatus required is simple, large volumes of cells can be handled easily, and the synchronous populations can be prepared within a few hours. In principle, the method should be applicable to any cell population in which phagocytosis occurs discontinuously over the cell cycle.

INTRODUCTION

The prime motivation for developing the large-scale method of synchronization described in this paper was to obtain sufficient cells to permit isolation of specific membranes and organelles for both physical-chemical studies and chemical characterization. Our interest in the properties of membranes isolated at various stages in the cell cycle was due to the fact that several presumably membrane-regulated phenomena change through the cell division cycle of *Tetrahymena*, e.g. phagocytosis (Chapman-Andresen & Nilsson, 1968) and swimming velocity (Hill & Hill, 1985), as well as the process of division itself. Our goal was therefore to determine whether these physiological processes are in any way connected with changes in the composition and physicochemical properties of the relevant membrane systems.

Some years ago, Zeuthen (1971), developed his well-known system for heat-induced synchronization of *Tetrahymena*. This method has, however, several disadvantages. First, it leads to the formation of synchronous populations that are abnormal in many respects, e.g. greater cell size (Zeuthen, 1971), lack of a G_1 period (Andersen *et al.* 1975) and probable perturbations of the lipid metabolism (Baugh & Thompson, 1973); second, the method is lengthy, requiring six or seven generation times in order to achieve synchrony; third, the method requires specially constructed apparatus and the correct heat-shock temperatures have to be established

Key words: *Tetrahymena thermophila*, synchronization, cell cycle, computer simulation.

empirically. Finally, although the heat-shock method has been successfully applied to *T. pyriformis*, little success has been had with other species of *Tetrahymena*, e.g. *T. thermophila* (Suhr-Jessen, 1978).

In addition, we felt that a selection method of synchrony was preferable to a more perturbing inductive method such as heat-shock, so we decided to try and develop the synchronization method based on the well-known observation that *Tetrahymena* do not phagocytose for a relatively short period before and after cell division (Nilsson, 1976). This concept was first presented for synchronization by Hildebrandt & Duspiva (1969), but the means of separating non-phagocytosing cells from phagocytosing cells was unsatisfactory.

In a subsequent development of the technique, separation of the non-phagocytosing cells was carried out using small glass columns, filled with a mixture of quartz and iron powder, clamped between the two poles of a pair of magnets (Dickinson *et al.* 1976). However, these columns have very slow flow-rates and are therefore not suitable for separations of more than 50 ml of cells.

In the method described in this paper, a number of simple but essential modifications have drastically improved the utility of the method, permitting synchronization of up to 12-l cultures of *Tetrahymena*.

MATERIALS AND METHODS

Cultures and growth conditions

Cultures of *T. thermophila*, B1868, Chx-2/Chx-2 (cysen, IV), hereafter referred to as *Tetrahymena*, were grown in 12-l batches in New Brunswick Microferm fermentors (New Brunswick, NJ) at 30·0 (±0·05) °C with aeration at 3·7 l min^{-1} and vane rotation at 140 rev. min^{-1}; 100 μl of Dow Corning Antifoam A was added per 12 l of medium to prevent excessive foaming. The growth medium consisted of 1% Bacteriological Peptone (Oxoid L37) and 0·1% yeast extract (Oxoid L21) to which $FeCl_3$ was added to a final concentration of 36 μM.

Synchronization procedures

The synchronization apparatus (Fig. 1) consisted of a plexiglass support stand in which four (41 cm) lengths of autoclavable Nalgene 8000 tubing (38·1 mm internal diam., 6·4 mm wall), filled with washed steel wool, were clamped between 8 pairs of ferrite magnets (131 mm × 51 mm × 17·5 mm anisotropic magnets, Cat. no. BM 95056, obtained from Bakker Madava magnetic systems, Reebommel 2, 5691, The Netherlands). The steel wool (grade 00, Thamesville Metal Products, Thamesville, Ontario, Canada), was washed extensively in ethanol and air dried overnight. Each column was filled for 2/3 of its length with a total of about 50–60 g of the washed steel wool, and then connected to the top reservoir and the collecting manifold. The column assembly was placed on top of a 6 mm spacer, which rested on the banks of magnets inserted into half the column support, and the other half of the support with its spacer and magnets was clamped down over the columns so that the short cross-section of the columns was about 38 mm, and the inter-magnetic distance 50 mm.

The colloidal ferrite suspension was made up as follows: 5 g of colloidal ferrite (0·05 μm diam. particles, type 4000, obtained from Wright Industries, Brooklyn, NY 11220) was suspended in 100 ml of 10 mM-Tris·HCl buffer, pH 8·0, containing 1·5 g bovine serum albumin (BSA; Sigma, fraction V). The suspension was then sonicated at room temperature for four 4-min periods each hour at 70% full power, using a 40% duty cycle, over a period of 3 h with a Branson model 350 Sonifier equipped with an 17·5 mm horn. The suspension was stored at 4 °C until required, and just before use was re-sonified for two 4-min periods as above.

For synchronization, cells were grown to a cell density of $1{\cdot}5\times10^5$ to $2{\cdot}8\times10^5$ cells ml^{-1}. For a single-step synchronization, 48 ml of the ferrite suspension were added to the cell culture in two portions of 24 ml or three portions of 16 ml at equal time intervals over the required period of time (typically 7–9% of the mean doubling time, t_d). At the end of this period the cells were transferred

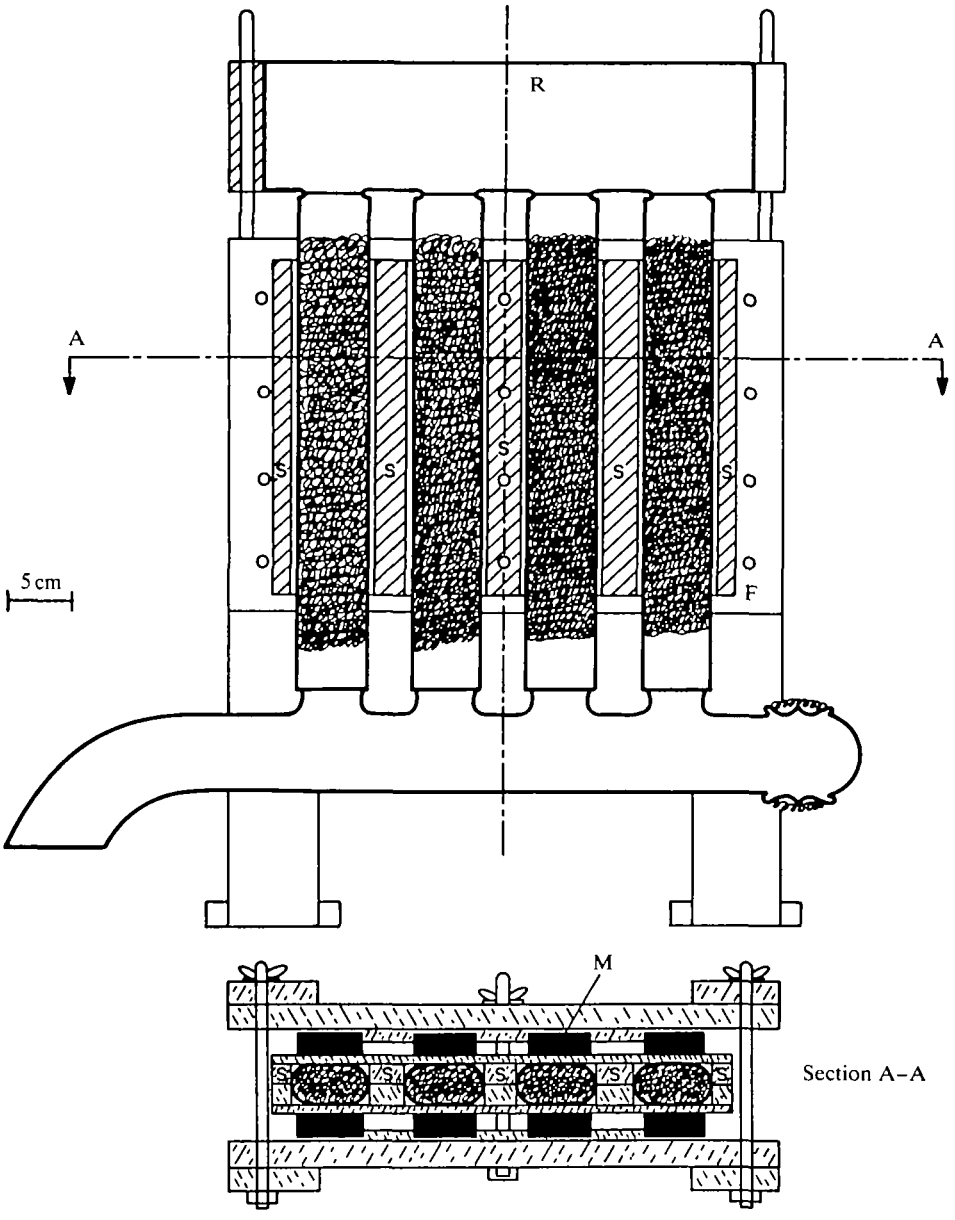


Fig. 1. Side elevation (top) and cross-section (below) through synchronization apparatus. In the side elevation the passage through which the cells flow is heavily outlined to indicate the reservoir (R) the columns, the spacers (S) and the collecting duct. The main supporting frame is marked F. In the cross-section through the middle of the apparatus the banks of magnets (M) are indicated.

to the reservoir of the separator and collected within 2 min. Cells coming off the columns after this time were discarded. For a two-step synchronization, the cells coming through the separator were placed in a clean fermentor, and growth continued. At exactly one doubling time (t_d) from the initial ferrite addition, ferrite was added again in the same batchwise manner as before. The degree of synchrony (F; according to Edwards & Lloyd, 1980) was calculated either in terms of the standard deviation of the population around the mean of the log-normal distribution of generation times (t_g), or more simply using the formula of Edwards & Lloyd (1980).

Cell counts were determined using a Coulter counter, model ZM, fitted with a 100 μm orifice. Duplicate 2-ml samples of cells were fixed in 2 ml of formaldehyde (4%, v/v) and diluted with 16 ml 0·4% NaCl 10 min prior to counting.

The six morphological stages were scored as described by Nilsson (1976), and their life-spans expressed as a percentage of the cell cycle, calculated according to Nachtwey & Cameron (1968).

All calculations were carried out on a microcomputer using programmes written in the biomathematics section of this division. Details of the calculations can be provided on request.

RESULTS

Throughout the text, we will refer to morphological stages I–VI, which have previously been described by Nilsson (1976).

Initially, selection synchrony was carried out on a small scale using 50-ml cultures of *T. thermophila*, and permitting cells to ingest ferrite for 12–14 min. After column passage, the degree of synchronization was typically only about 60–70%, as judged by the percentage of stage I (daughter cells) in the population. Since it proved impossible to obtain reproducibly good synchrony we decided to determine which parameters were important for the success of the synchronization procedure. These are discussed individually below.

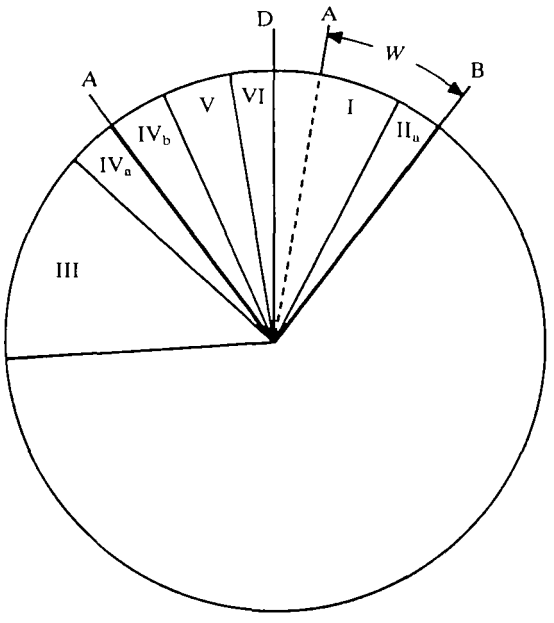
Age distribution of the selected population-wedge size

When ferrite is added to a population of *Tetrahymena* and left for about 10 min, most of the cells showing a cleavage furrow, as well as the newly formed daughter cells, do not phagocytose. These cells, which pass through the magnetic separator, have an age distribution that depends on the extent of the non-phagocytic period and on how long the cells have been ingesting ferrite. The limits of this age distribution will be referred to as the wedge size (W, as % of t_d; see Fig. 2). The wedge size, together with the distribution of generation times (σ), will determine both the degree of synchrony of the selected population and the overall yield of cells passing through the separator. In order to determine the wedge size we first had to determine the start and finish points of the non-phagocytic period in the cell cycle. This we did in two ways.

In the first method the ferrite suspension was added to the cell culture at zero time, and samples were withdrawn for fixation in formaldehyde at 1–5 min intervals over the first 25–40 min. Examination of fixed cell samples under the microscope showed that during the first 5–10 min, the food vacuoles were densely packed with ferrite. After a further 5 min, the newly formed vacuoles were seen to contain far less ferrite, and by 20–25 min the vacuoles were nearly always devoid of ingested material. This dearth of suitably fine particulate material after a period of 10–15 min is due to the very rapid filtration rate of *Tetrahymena* (Nilsson, 1976; Rasmussen, 1976). We

subsequently added the requisite amounts of ferrite in two or three portions so that particulate material was always available for ingestion. In the samples taken for the determination of the non-phagocytic period, the percentage of stage IV cells that had ingested ferrite was scored in the first four or five samples, and this percentage was plotted against time (see Fig. 3A). From this curve, the point in time at which stage IV cells ceased to phagocytose can be calculated. Subsequently, at least 1000 cells were counted in order to determine the distribution, and thus the age limits of the six morphological stages. Knowing when in stage IV phagocytosis ceased, and the age span of stages V, VI and I, we needed to determine only at which point in time stage II cells started to phagocytose in order to define the limits of the non-phagocytic period. This was done by plotting the percentage of stage II cells that phagocytosed against time (see Fig. 3B).

In the second method, which required less counting time but was not as accurate as the first method, the start point of the non-phagocytic period was determined as before, and the total length of the non-phagocytic period was found by plotting the percentage of all cells with ferrite against time (see Fig. 3C). Typical results obtained from the two methods are shown in Table 1. It should be stressed that the distribution of morphological stages can change significantly when culture conditions are altered. This of course implies that both the stage distribution and the length of the non-phagocytic period must be re-determined whenever any factor affecting growth is altered, e.g. O_2 availability, temperature, pH etc.



I	7·4 ± 1·8	10·2 %
II_a	2·8 ± 0·1	
II_b	63·8 ± 4·1	
III	12·8 ± 1·7	
IV_a	2·8 ± 1·0	
IV_b	3·6 ± 1·3	10·41 %
V	4·1 ± 0·9	
VI	2·7 ± 0·8	

Fig. 2. Representation of the age spans of the various morphological stages based on the mean values of seven determinations of the stage distribution. In all, 12665 cells were counted. The non-phagocytic period thus extends from A to B, i.e. from approximately the mid-point in stage IV to about 5% into stage II. The wedge size (*W*) is represented by A′ to B when cells have phagocytosed for a period of time corresponding to A to A′.

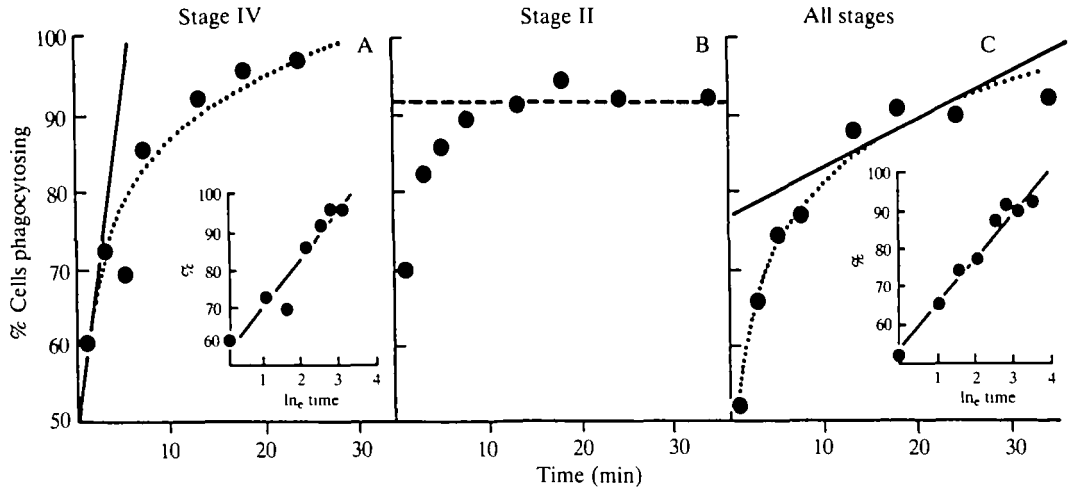


Fig. 3. Determination of the limits of the non-phagocytic period. A. The percentage of stage IV cells that have ingested ferrite is plotted against time, enabling the age limits of stage IVa, and hence the start-point of the non-phagocytic period to be determined. B. A similar plot is illustrated for stage II cells, from which the length of stage IIa and thus the end-point of the non-phagocytic period can be determined. C. A plot of the percentage of all cells that have phagocytosed against time, which provides an alternative method of determining the end point of the non-phagocytic period. The insets show the same data, as log functions.

From Table 1 it can be seen that the length of the non-phagocytic period is close to 21% of the cell cycle under normal experimental conditions (30°C, cell density 2×10^5 cells ml^{-1}), but decreases by about 10% when the growth temperature is increased to 38°C. There is no apparent correlation between the non-phagocytic period and the cell doubling time t_d. Thus, with a knowledge of the doubling time,

Table 1. *Length of the non-phagocytic period*

Culture density ($\times10^3$ ml^{-1})	Culture temperature (°C)	t_d (min)	Non-phagocytic period (% t_d)	
			Method 1	Method 2
95	30·1	131·2	19·05	19·34
100	30·0	125·9	19·58	19·03
106	31·4	125·9	—	17·96
137	30·0	158·4	25·23	27·90
191	30·4	186·8	19·49	—
210	30·1	138·1	22·52	19·78
222	30·0	167·4	23·03	22·43
280	30·3	304·5	22·50	18·54
	Mean values ± S.D.		21·63 ± 2·13	20·71 ± 3·22
162	38·1	116·0	20·63	—
243	38·4	142·7	19·51	—
270	38·7	156·9	19·10	—
	Mean values ± S.D.		19·75 ± 0·65	

t_d is the mean doubling time of the culture.

determined prior to the addition of colloidal ferrite to the culture, both the length of the non-phagocytic period and the wedge size corresponding to a given period of contact with ferrite can be calculated.

On the expectation that the degree of synchrony would improve as the wedge size was decreased, a series of synchronizations were performed in 12-l cultures, in which the ferrite contact time was varied so as to give wedges from 9·5 to 2·1 % of t_d. Somewhat surprisingly, the degree of synchrony (F), as measured according to Edwards & Lloyd (1980), was unaffected by a decrease in the wedge size (see Fig. 4).

Yield of cells in the selected population

Since the synchrony obtained when using even very small wedges was not as good as anticipated, we measured the yield of cells obtained after passage of the population through the separator. The expected yield was calculated from a knowledge of the cell density and the percentage of cells without ferrite in each of the six

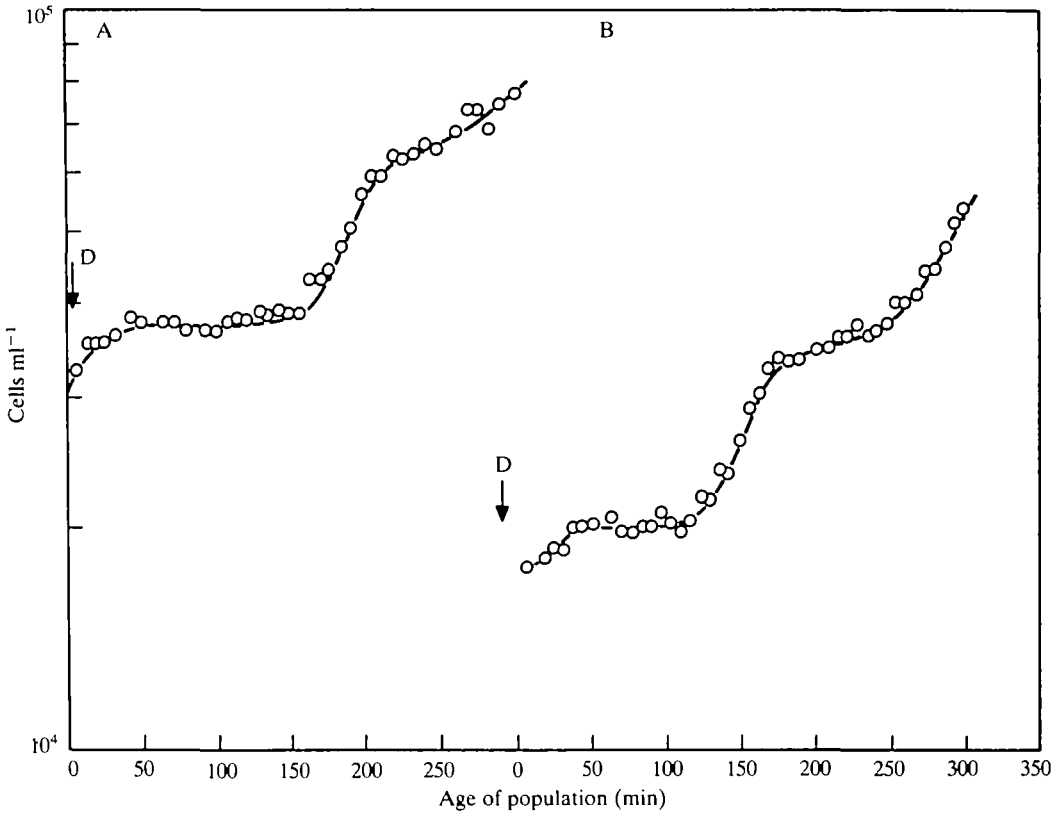


Fig. 4. The effect of changing the wedge size on the degree of synchrony. Cultures of 12 l were synchronized once as described in the text. In A, $W = 13{\cdot}2\% t_d$, and the degree of synchrony, F, was 0·51. t_d was 177·4 min prior to synchronization. In B, $W = 6{\cdot}83\% t_d$, $F = 0{\cdot}50$ and t_d was 145·5 min prior to synchronization. In both figures the point at which the first division occurred is marked with an arrow (D). Note that although the wedge size has been halved, the degree of synchrony remains unchanged.

morphological stages just prior to column passage. When due account is taken of cell division during the few minutes required for column passage etc., the ratio of the calculated cell density to that expected gives the actual yield.

The first cell yield determinations showed that typically between two and three times the expected number of cells passed through the separator. In a subsequent synchronization experiment, 1·2-l fractions were collected from the bottom of the separator and the cell yield in each fraction was determined (see Table 2). Examination of the cells in the later fractions showed that besides the expected stage I and stage IIa cells, there were significant numbers of cells from later stages. This suggested that the ferrite was somehow being removed from many of the cells, and that this process only took a few minutes.

This conjecture was verified by the following small-scale synchronization experiments. Ferrite was added to two 1-l cultures so that there was an approximately 1% t_d wedge; in the first culture the cells were passed through the column immediately; in the second, a pair of magnets was placed directly underneath the culture flasks for 4 min before column passage. During the 4 min for which the latter culture was subjected to the magnetic field, the percentage of cells without ferrite rose from 0·76 to 13·12% of the total cells. As a consequence, whereas in the untreated control the density of the synchronized population was 2·1% of the original culture density, in the culture subjected to strong magnetic fields some 14·5% of the cells passed through the separator. Photographs taken at 1-min intervals during subjection of the

Table 2. *Yield of cells passing through magnetic separator*

Fraction number	Cell density ($\times 10^3$ ml^{-1})	% Cells passing through separator	Yield
1	36·35	11·87	1·04
2	38·23	12·48	1·10
3	42·19	13·77	1·21
4	55·42	18·09	1·59
5	54·60	17·83	1·57
6	60·98	19·91	1·75
7	64·22	20·97	1·85
8	70·77	23·10	2·03
9	268·4	87·62	7·71

A 12-l culture of *T. thermophila* was grown at 30·35°C to a density of 280×10^3 cells ml^{-1} (t_d = 146 min), and ferrite added in three 16-ml batches over a period of 15 min. Immediately before separation of the cells on the magnetic columns, a sample was fixed for determination of the percentage of cells without ferrite. During passage through the separator (using 6 mm spacers and an inter-magnetic distance of 50 mm), nine fractions were collected for yield determinations. The wedge size was 10·8% of t_d, and therefore the selected population consisted primarily of newly divided cells.

The first eight fractions had a volume of 1·2 l, the last fraction was 2·1 l.

The density of the culture immediately prior to passage was $306\cdot3\times10^3$ cells ml^{-1}.

Yields were calculated as follows: cells without ferrite constituted 11·36% of the original population: thus, the expected cell density was $306\cdot3\times0\cdot1136 = 34\cdot80\times10^3$ cells ml^{-1}, and the yield for the first fraction was therefore 36·35/34·80 = 1·04.

cells to the magnetic field showed that within 30 s the ferrite-containing food vacuoles had aligned into rods, and that over the next 2 min many of these rods were drawn out through the outer membrane system.

Following these observations, we carried out a series of experiments in which the distance between the magnets on the separator was varied systematically. For the magnets used in our separator, we found that an inter-magnetic distance of 50 mm was optimal when using 6 mm plastic spacers. This gives a magnetic field strength of between 0·3 and 0·4 tesla.

Ferrite concentration

We have tested a wide range of ferrite concentrations and found that with the above inter-magnetic distance and spacer thickness, a final concentration of 200 mg ferrite l^{-1} culture is close to optimal. At lower ferrite concentrations, many of the food vacuoles contained little ferrite, and as a consequence many cells passed through the separator. At higher ferrite concentrations, there was an increasing tendency for food vacuoles to be pulled through the outer membrane system by the magnetic field.

Flow rates

Since the complete withdrawal of ferrite-containing vacuoles occurred on a time scale of a few minutes, we packed the steel wool into the columns as loosely as possible, so that the culture could flow through the separator at the maximum rate (7 l min^{-1}). The cells emerging from the separator after 2 min were mostly non-synchronous, and were always discarded.

Distribution of generation times

As stated previously, the wedge size, contrary to expectations, had little influence on the degree of synchrony. We therefore set up a computer simulation of how an initially synchronized culture would be expected to behave with time. For this we made the following assumptions: (1) that the generation times are log-normally distributed (Nachtwey & Cameron, 1968), with the standard deviation usually in the range 0·1 to 0·2 h; (2) that part of the inherent asynchrony of a selected population is due to the age distribution within the wedge; and (3) that the life spans of the morphological stages I to VI are a constant percentage of t_d, whatever their numerical values.

Some of the simulations are shown in Fig. 5. These simulations confirmed the experimental observation that decreasing the wedge size had little effect on the decay of synchrony, as judged by the similar distributions of stage VI with time (compare Fig. 5A and B). However, the effect of varying the distribution of generation times, σ, was dramatic (see Fig. 5A,C,D). These simulations demonstrate that the distribution of generation times is probably the single most important factor in determining how rapidly the synchrony of a selected population of cells decays.

Effect of a second synchronization step

Studies with *Tetrahymena* (Schafer & Cleffmann, 1982) and other species (Brooks, 1981), have shown that the generation times of daughter cells are more closely correlated than those of other cell pairs randomly chosen from the population. Thus, cells with a generation time close to the median value would be expected to produce progeny with generation times deviating less from the median value for the culture than would the progeny of cells having extreme values of the generation time. It follows that a greater degree of synchrony could in certain circumstances be achieved by imposing a further selection step on an already synchronized population. This second ferrite addition should be timed so that cells with longer and shorter generation times than the median for the population are eliminated.

Initially, the timing of the second ferrite addition was decided upon after examination of the cells under a microscope, and ferrite was added when there was an

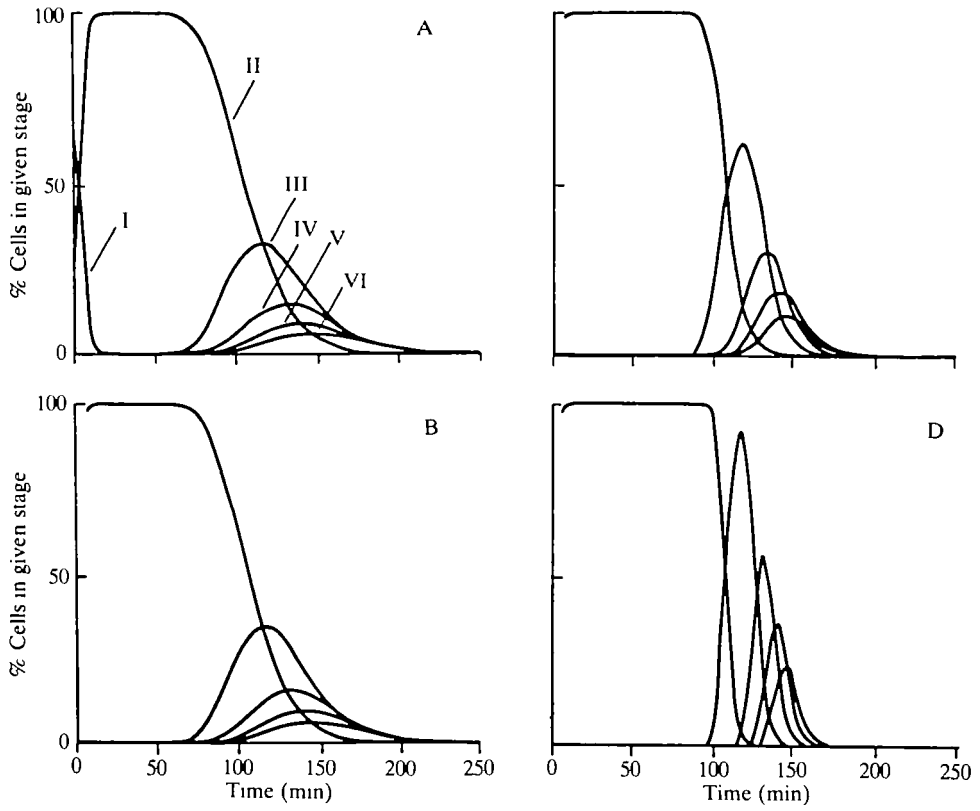


Fig. 5. The effect of the wedge size (W) and the distribution of generation times (σ) on the decay of synchrony of a synchronous culture. In these computer simulations, t_g was taken as 150 min, and the morphological stages had the following life spans expressed as a % of t_g: I, 7·41; II, 66·72; III, 12·75; IV, 6·40; V, 4·07; VI, 2·65. In A and B, σ has been kept constant at 0·20, and W varied from 10·12 % t_d in A to 2·22 % t_d in B. In C and D, σ has been decreased to 0·10 and 0·05, while keeping $W = 2{\cdot}22\,\% t_d$. The effect of varying σ is marked, in contrast to the small effect seen when W is changed (compare with the experimental observations shown in Fig. 4).

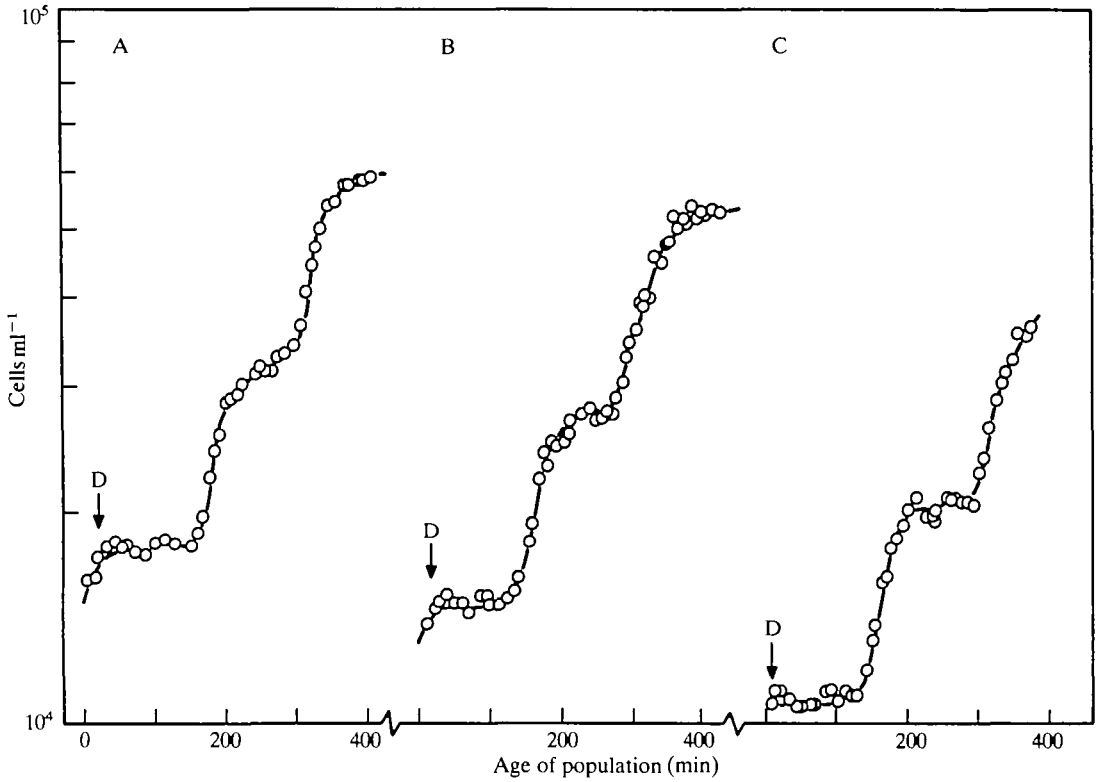


Fig. 6. The effect of varying the wedge width in the double synchronization procedure; 12-l cultures of *T. thermophila* were grown at 30·4°C to densities of: A, $1{\cdot}32\times10^5$ cells ml^{-1}; B, $1{\cdot}94\times10^5$ cells ml^{-1}; and C, $2{\cdot}68\times10^5$ cells ml^{-1}, and thereafter subjected to two identical synchronization steps spaced one generation apart. The wedge size was 15·7% in A, 14·4% in B and 11·7% in C. The degree of synchrony was seen to increase as the wedge size and the yield decreased. For A, $t_d = 149{\cdot}6$ min, $y = 12{\cdot}85\%$ and $F = 0{\cdot}60$. For B, $t_d = 163{\cdot}9$ min, $y = 7{\cdot}2\%$ and $F = 0{\cdot}62$; for C, $t_d = 148$ min, $y = 4{\cdot}1\%$ and $F = 0{\cdot}77$. D marks where the first division occurred.

apparent peak in stage IV cells. In subsequent experiments it was shown that the best degree of synchrony was obtained when the ferrite addition was made at exactly one doubling time (t_d) from the first.

The double synchronization procedure worked well. As seen in Fig. 6, in contrast to the single synchronization procedure, the degree of synchronization attained depended on the wedge size used, with the best synchrony obtained at smaller wedge sizes. However, as the synchrony improved, the cell density of the selected population became lower. In the final version of the technique, a compromise was made, and a moderate wedge size ($W = 14\%\ t_d$) was chosen that gave both a reasonable synchrony ($F = 0{\cdot}62$) and a moderate yield (see Fig. 6B; note that the yield (y) is defined here as [cell density in the synchronous culture/original cell density]$\times100\%$).

As was the case with the single-step synchronization procedure, we observed that the yield deteriorated with increasing cell culture density. We believe that this

decrease in yield is due to a broadening in the distribution of generation times at higher cell densities. As the distribution broadens, an increasing number of cells (those with the slower growth rates) will phagocytose at the time of addition of the second batch of ferrite. Furthermore, a considerable proportion of the faster growing cells will also be eliminated during the ferrite contact period as they progress beyond stage IIa. The overall effect will be of reducing the distribution of generation times within the population at the expense of yield.

In a few double-synchrony experiments, the degree of synchrony was also assessed by scoring the percentage of the various morphological stages with time (Fig. 7). Note that as the population proceeds through the cycle, the widths at half peak height become progressively larger than would be predicted from the age data in Fig. 2. This broadening of the peaks is a reflection of the gradual breakdown in synchrony as

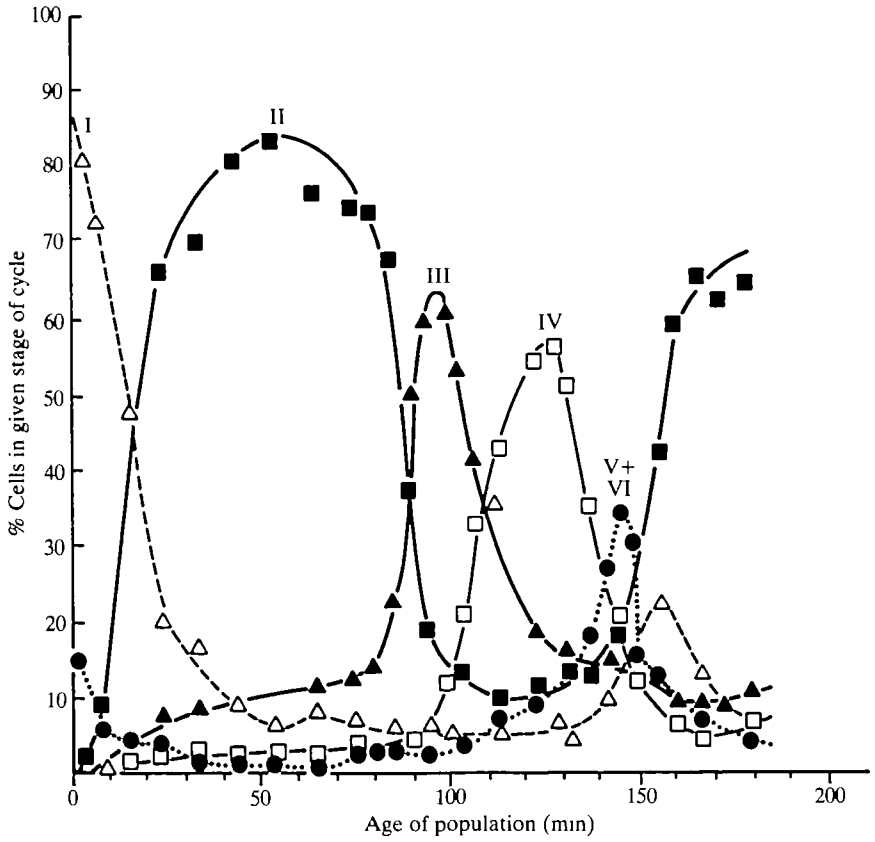


Fig. 7. The synchrony of *T. thermophila* was measured by scoring morphological stages. A 500 ml culture of *T. thermophila* was synchronized by the two-step procedure, samples were withdrawn at regular intervals, and the percentage of the morphological stages was scored. A comparison of the widths at half peak height with the data in Fig. 2 gives a good indication of how the synchrony decays through the cell cycle (see also the simulations in Fig. 5).

the cell population proceeds through the cell cycle (compare with the simulations in Fig. 5).

DISCUSSION

We have developed a simple method for the large-scale synchronization of *T. thermophila* based on the selection of young non-phagocytosing daughter cells. Two variants of the method have been used; in the first, cells are left in contact with a suspension of colloidal ferrite for a period of some 12–17% of the cell cycle time t_d. After separation on a magnetic column (separator), a population of acceptably high density and having a reasonable degree of synchrony is obtained. This single-step version of the method has the advantage of being quick, and the disadvantage that the synchrony of the selected population decays appreciably by the latter third of the cell cycle (see Figs 4, 5).

The second method involves leaving the cells in contact with ferrite for some 7% of t_d ($W = 14\%\ t_d$), separating the cells and then waiting one doubling time before repeating the ferrite treatment. If the ferrite is added sooner than this, many of the cells will not yet have reached the non-phagocytic stage, and a faster-growing population will be selected. If the ferrite is added later, e.g. at $1{\cdot}15 \times t_d$, many cells will already have reached division, and the faster-growing cells will be eliminated during the selection procedure, leaving a slower-growing population. When ferrite is added at exactly $1{\cdot}00 \times t_d$ the cell yield will be maximal, and the faster growing cells will be eliminated (as early stage IIb) as will slower-growing cells (as stage IVa), thus narrowing the distribution of the logarithms of generation times, σ. The double synchronization procedure gives a lower cell yield than the single synchrony method, but the synchrony is much better maintained (compare Figs 4 and 6). Under these conditions the population will consist primarily of stage I cells. In contrast, the single synchrony method gives a slightly older population of cells, often almost an equal proportion of stages I and IIa, and is thus not as suited to the study of events starting immediately after division as is the double synchrony method.

Most of the results published on the cell cycle of *Tetrahymena* have been obtained using the heat-shock method of synchronization. This method suffers from several disadvantages; the temperature regime has been shown to perturb lipid biosynthesis (Baugh & Thompson, 1973), as well as yielding cells of considerably larger volume than usual (Zeuthen, 1971). In addition, the timing of key events such as DNA replication and cytokinesis are altered by the heat-shock procedure. Thus, when cell-cycle-related changes, such as the variation of internal pH, are studied using the Zeuthen system, the temporal disposition of events is often different from that seen when using cells synchronized by a different method (Gillies & Deamer, 1979). Furthermore, thymidine incorporation studies have demonstrated that in heat-shock synchronized cultures, the cells enter the S phase immediately after division, and thus lack the G_1 phase of the cell cycle (Andersen *et al.* 1975). However, using the thymidine-pulse technique, we have been able to show that cells synchronized by our selection technique have a G_1 period of normal length (unpublished data).

Several other synchronization techniques have been described that are applicable to *Tetrahymena* (see Andersen *et al.* 1975, for a review). Amongst these are starvation/refeeding procedures (Cameron & Jeter, 1970) and hypoxic shock (Dickinson *et al.* 1977), neither of which yields as good a synchrony as achieved using our double synchrony procedure. In addition, these techniques have, to date, been applied only to small volumes of cultures.

The procedure described for double synchrony is now in routine use in our laboratory on a 12-l scale. Since we culture the cells under closely controlled conditions, it is not necessary to determine t_d or the length of the non-phagocytic period prior to each experiment. We use the mean values calculated from a previous series of experiments carried out under standard conditions. The synchronization is started in the morning by addition of the first batch of ferrite for 8% of t_d, so that approximately 2·5 h later 12 l of a synchronous culture are available for experimentation.

Owing to its simplicity, the method is amenable to both automation and scaling-up. Synchronizations of up to 50 l should be possible with only slight modifications. Synchronization of such large amounts of cells would permit isolation of sufficient quantities of membranes for nuclear magnetic resonance studies. As it is, the 12-l scale experiments described here have yielded enough membrane material from three points in the cell cycle for the simultaneous determination of order parameters using electron spin resonance probes (spin probes), and for lipid analyses (unpublished data). Because of the requirement for large amounts of membranes, these types of experiments have not previously been possible.

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