THE CELL CYCLE AND GLYCOLYTIC ACTIVITY OF SCHIZOSACCHAROMYCES POMBE SYNCHRONIZED IN DEFINED MEDIUM

by

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Keywords: Cell cycle, Schizosaccharomyces, heat shocks, synchrony, glycolysis

Schizosaccharomyces pombe was grown in a chemically defined medium (EMM 2). Cell cycle synchrony was induced with 5 or 6 heat shocks (60 min, 41°C) spaced a normal cell generation apart (140 min, 32°C). The sequence of nuclear division, cell plate formation, DNA synthesis and cell division is normal, and both DNA and cell number double in each cycle.

The glycolytic activity was also followed. It was found that the rate of glycolysis increased linearly between the synchronous divisions with doubling of the rate of increase at each division.

1. INTRODUCTION

It has previously been shown by KRAMHØFT and ZEUTHEN (8,9) that division synchrony can be induced in Schizosaccharomyces pombe by a controlled temperature cycle. Cultures in exponential growth are treated with a series of heat shocks spaced one generation time apart. Within discrete time intervals between successive shocks the number of cells doubles. After cessation of the heat shocks two synchronous divisions follow. The cell cycle is close to normal after induction of synchrony by

heat shocks. This is shown by KRAMHØFT, NISSEN and ZEUTHEN (7) who compared the heat shock induced cell cycle with the supposedly normal cell cycle obtained with the selection procedure of MITCHISON and VINCENT (15). Nuclear division, cell plate formation, DNA synthesis and cell division (fission) are spaced at nearly normal intervals in heat synchronized cultures.

The glycolytic activity of *S. pombe* increases linearly between successive cell divisions, and the rate of increase in activity doubles at

the time of each division, as shown by HAMBURGER, KRAMHØFT, NISSEN and ZEUTHEN (5). This pattern is observed in cultures after selection synchrony, after induction of synchrony by heat and in clones displaying natural synchrony.

These studies were all carried out with cells growing in a complex medium. In the present work we demonstrate induction of synchrony by heat shocks in cultures grown in a chemically defined medium. As previously observed for cells in complex medium we find that the cell cycle after induction of synchrony by heat is close to normal.

2. MATERIALS AND METHODS

Stock cultures of the haploid strain 972 h⁻ of *Schizosaccharomyces pombe* were kept at 4°C on slanted agar medium containing yeast extract and glucose as described previously (7). Exponentially multiplying "starter cultures" produced as described in (7) were used in all cases. Except when otherwise stated, the medium used in the present report was a chemically defined medium, "Edinburgh Minimal Medium", EMM 2, which contains glucose, a nitrogen source, salts, vitamins and trace elements (12). Whenever the text refers to a complex medium, this is the yeast extract-glucose medium, YEG, used by us in previous work (5, 7, 8, 9).

Cultures were grown in Erlenmeyer flasks with magnetic stirring. Heat synchronized cultures were prepared following the principles published in refs. (7, 8). For further details of the present synchronizing treatment see the Results section.

In a few experiments cultures were synchronized by the selection technique of MITCHISON and VINCENT (15) using lactose gradients (15-30 per cent) and keeping the temperature at 32°C during the selection procedure.

Cell numbers were calculated from counts in a Bürker-Türk counting chamber. Nuclei were stained with Giemsa according to GANESAN and ROBERTS (3) and GANESAN and SWAMINATHAN (4).

DNA was assessed by the diphenylamine

method modified as suggested by MITCHISON and CREANOR (personal communication detailed in (7)). Total RNA was measured as the extinction at 260 nm after extraction with hot perchloric acid, and protein was determined according to the method of LOWRY et al. (11).

Gaseous exchanges (uptake of O₂ and production of CO₂) were assessed using both the gradient diver technique (6, 21) and the Warburg technique (19). Details of these methods when applied to cultures of *S. pombe* have been described elsewhere (5).

3. RESULTS

3.1. Heat shock synchrony

A cyclic heat treatment can be used to synchronize cultures of *S. pombe*. The principles and considerations behind the elaboration of the cyclic heat program have been discussed in detail elsewhere (7, 8). In short, synchrony is achieved when cultures grown under optimal conditions (YEG) are treated with heat shocks spaced one generation time apart, provided the temperature and the duration of each shock are chosen according to prescription.

Results published so far all originated from experiments with cells grown in YEG, the exact chemical composition of which is unknown. One aim of the present report has been to establish heat shock synchrony in a *chemically defined medium*, EMM 2. Since *S. pombe* grows at a slower rate in EMM 2 than in YEG the details of the synchronizing treatment had to be reconsidered.

The variable parameters of the heat shock program are, 1) the duration of each heat shock, 2) the shock temperature, 3) the interval between successive heat shocks, 4) the temperature between shocks, and 5) the number of shocks sufficient to give satisfactory synchrony.

When heat synchrony in *S. pombe* was originally developed, these parameters were chosen rather fortuitously. However, it was demonstrated that all parameters, except for the duration of each shock, appeared to be well chosen. We then stated that the duration of each shock (parameter 1) might have been chosen too short. This statement was based on the observations in Fig. 1 A, which shows ef-

Figure 1. Delay and »set-back« of a division as a function of the duration of the final heat shock. Frame A shows results from cells synchronized in YEG and is redrawn from Fig. 7, ref. (8). Frame B shows results from cells synchronized in EMM 2.

fects on the forthcoming division of variations in the duration of a heat shock (data from (8)). The cultures had been synchronized with 6 standard heat shocks (30 min) the duration of the seventh shock was varied, and the time of the forthcoming division was determined. It is seen that a heat shock causes a delay of the division which increases with shock length (curve a). Furthermore, the division was delayed in excess of the duration of the shock. This excess delay, or »set-back«, is plotted in curve b as a function of the duration of the shock. The »set-back« also increased with increasing shock length but only to a point after which further increase in shock duration caused no further »set-back«. A maximal »set-back« of about 40 min was seen with shocks lasting 50 min or more.

According to observations with heat synchronized cultures of the ciliate protozoon *Tetrahymena pyriformis*, best synchrony is achieved when the »set-back« is maximal (20). Therefore the duration of each shock

(parameter 1) was chosen to be 60 min according to the set back curve in Fig. 1 A.

Following previous observations, temperature (parameters 2 and 4) were kept at 41°C and 32°C during shock and intershock periods, respectively (7, 8). The generation time of *S. pombe* in EMM 2 is 140 min at 32°C (cf. Table I). Thus the intershock interval (parameter 3) was chosen at 140 min.

This heat shock program results in satisfactory synchrony as demonstrated in Fig. 2. The upper frame shows variations in cell number as a function of time after 5 heat shocks after which time the cyclic heat program was discontinued. Thus the figure shows a culture in free running synchrony. It is seen that the heat treatment results in two bursts of synchronous divisions each causing a doubling of the number of cells in the culture.

Synchrony was also monitored as the variation with time in the cell plate index, the fraction of cells with cell plates. This is shown in the lower frame, which demonstrates that

Figure 2. Variations in cell number (cells·ml⁻¹) and cell plate index as a function of time after the end of heat treatment (EH). Upper frame: cell number, lower frame: cell plate index.

each doubling in cell number is preceded by a peak in the cell plate index.

A series of experiments were then conducted to evaluate if the chosen parameters were optimal, and if not, whether synchrony could be

improved by changes in one or more parameters.

To check parameter 1, the duration of the heat shock, delay and »set-back« curves like the ones shown in Fig. 1 A (YEG medium) were constructed for cells synchronized in EMM 2. Cultures were treated with six 60 min heat shocks and the duration of the seventh shock was varied. The results are shown in Fig. 1 B. It is seen that also in EMM 2 the »set-back« is maximal (about 45 min) when the shock exceeds 50 min. Thus there is striking similarity in response to a temperature shock in cells synchronized in YEG and EMM 2. Accordingly, the choice of 60 min as the duration of each heat shock in the present work was a good one.

Reduction of the shock duration to 45 min resulted in somewhat inferior synchrony. The *restricted* synchrony, i.e. the synchrony of cultures which continue to be exposed to the cyclic heat treatment, is satisfactory (cf. Fig.8), but the synchrony of the second free running division is poor. Reduction of the shock temperature (parameter 2) to 40°C also resulted in poorer synchrony. So did change of the intershock interval (parameter 3) to 135 or 145 min. Parameter 4 (intershock temperature) was not varied in these experiments. The number of shocks sufficient to induce synchrony (parameter 5) was optimal at 5 or 6 heat shocks which gave equally good synchrony.

In agreement with observations presented above we have settled on the following cyclic

Table I

Time points of 1st and 2nd synchronous division in heat synchronized cultures of *Schizosaccharomyces pombe*.

	Defined medium (EMM2)	Min after EH Complex medium ¹⁾ (YEG)
a: 1 st division	99 ± 6 ²⁾ (n = 32)	94 ± 7 (n = 32)
b: 2nd division	198 ± 9 (n = 22)	186 ± 12 (n = 8)
b-a: Free running generation time	99 min	92 min
Generation time of exponentially growing cells	138 ± 7 (n = 11)	107 ± 5 (n = 24)

¹⁾ Values from ref. (6).

²⁾ Standard deviation.

heat program for induction of synchrony in *Schizosaccharomyces* in EMM 2: five or six heat shocks spaced apart by 140 min at 32°C and each lasting 60 min at 41°C.

The time of the occurrence of the synchronous divisions (the times of 50 per cent increase in cell number above plateau values) are given in Table I. For comparison we show equivalent data from cultures which have been heat synchronized in YEG. The table indicates that even though the generation time of exponentially multiplying cells in EMM 2 is prolonged by about 30 min compared to cells growing in YEG, the two synchronous divisions occur at identical times relative to the termination of the heat treatment. Consequently, in the two systems the generation times of free running cultures are identical, and about 95 min.

3.1.1. Nuclear division and cell plate formation

The time of nuclear division was estimated on Giemsa stained preparations. Three morphological groups can be distinguished, i) cells with one nucleus, ii) binucleate cells without a visible cell plate, and iii) binucleate cells with cell plate. In synchronous populations the percentage of each of these groups changes with time as the culture progresses towards cell division.

In Fig. 3 (lower curve) we show the variation with time in the percentage of binucleate cells without cell plate (filled circles), of binucleate cell with cell plate (triangles) and of the sum of these two fractions, (open circles). Also the variation with time in the number of cells in the culture is indicated (upper curve). The figure shows that prior to the first division there is a peak of about 16 per cent of cells with two nuclei and without cell plate (filled circles). As cell plates appear, this fraction decreases, but the sum of cells with two nuclei (open circles) increases much and passes a sharp peak (at about 60 per cent) as a consequence of cell division.

The interval between nuclear division and cell division was calculated in the following way: the times of equal percentage increase (40%) in the sum of cells with two nuclei and in cell number was read from the curves as in-

dicated by the level of the dotted lines. From the curves we read the difference between the times when the population passes 40% nuclear

Figure 3. Upper frame: variation with time after EH of cell number ($\text{cells}\cdot\text{ml}^{-1}$); lower frame: variation with time in the percentage of binucleate cells without cell plate (filled circles), binucleate cells with cell plate (triangles) and the sum of all binucleate cells (open circles) in stained preparations, as a function of time after EH.

The dotted lines mark 40 per cent increase in the sum of binucleate cells, cells with cell plates and cell number.

division and 40% cell division, and we accept this as a measure of the time which it takes the average cell to progress from nuclear to cellular division. This was found to be 22 min.

The time from cell plate formation to cell division was calculated analogously, and it was found that cell plates were formed 13 min prior to the first division in the average cell. From experiments in which variation in the percentage of cells with cell plate was followed on living, unstained cells it was observed that the cell plate was formed 12 ± 4 min ($n=8$) prior to the first division, and 14 ± 10 min ($n=4$) prior to the second synchronized division. These results are in good agreement with the equivalent data from selection synchronized cultures in EMM 2 and YEG.

3.1.2. DNA, RNA and protein

As described above, treatment with heat shocks induces two free running synchronous divisions. Each of them is preceded by a period of DNA synthesis as shown in Fig. 4, curve a. For practical reasons only one DNA synthetic period was followed in each single experiment,

Figure 4. Variations with time after EH in DNA·ml⁻¹ (curve a), protein·ml⁻¹ (curve b) and RNA·ml⁻¹ (curve c). The arrows indicate the time points of the synchronous divisions (50 per cent increase in cell numbers) in the respective experiments, and the ordinate indicates doublings in the content of DNA, RNA and protein·ml⁻¹ (arbitrary units).

and this is indicated by the choice of separate symbols. The time of 50% increase in DNA content precedes both divisions by 25 min. The divisions are indicated by the arrows. However, in other EMM 2 experiments we have found mid-S at later times and as late as about 5 min prior to each of the two divisions.

Usually the doubling time for DNA exceeds the doubling time for cell numbers by about 15 min and this should measure the duration of the S-period in the average heat synchronized cell. This value is in excellent agreement with results published by MITCHISON and CREANOR (13) for selection synchronized cells.

Fig. 4 also shows the increase with time in protein (curve b) and RNA (curve c). It is seen that both RNA and protein increase continuously during the two synchronous divisions (midpoint indicated by arrows). During the first 30-45 min after the end of a heat shock synthesis of both protein and RNA is somewhat suppressed.

3.1.3. Conclusions

The time sequence of cell cycle specific events in *S. pombe* is shown in Table II. In the first column are indicated the times by which nuclear division (ND), DNA synthesis (S) and cell plate formation (CP) precede cell division in cultures synchronized by heat shocks in EMM 2. We see no significant difference between heat synchronized cells and selection synchronized cells (column 2). Neither do we see differences which can be referred to choice of medium, EMM 2 (columns 1 & 2) or YEG (columns 3 & 4). It should be noted however, that the timing of DNA synthesis relative to cell division is subject to some variation.

3.2. Glycolysis

The glycolytic activity of exponentially and synchronously multiplying cultures was estimated using the gradient diver (5, 6, 21). A few hundred cells representing a mass culture were isolated in ampulla divers which were placed in linear density gradients. The divers were set afloat in the gradients 1.5 h before their upward migration – a measure of the glycolyte rate – was recorded by photography (6). Simulta-

Table II

Time points of occurrence of nuclear division, DNA synthesis and cell plate formation.

	Minutes prior to division							
	EMM2				YEG			
	1. Heat shock div. 1	div. 2	2. Selection ¹⁾ div. 1	div. 2	3. Heat shock ²⁾ div. 1	div. 2	4. Selection ³⁾ div. 1	div. 2
Nuclear division (ND)	25	–	35	–	35	–	30	30
DNA synthesis (S)	5 25	5 25	5	–	14	16	5	–
Cell plate form. (CP)	14	14	20	–	20	–	20	20

¹⁾ Data from MITCHISON & CREANOR (13) and BOSTOCK (1).²⁾ Data from KRAMHØFT, NISSEN & ZEUTHEN (7).³⁾ Data published as a student thesis (17).

neously, the growth of the mother cultures was followed by cell counts. As pointed out earlier, the upward migration of a diver reflects the difference between oxygen taken up from, and CO₂ released into, the diver's gas space (5). With the Warburg technique (20) the CO₂ production of cells in defined medium was 13.6 ± 0.6 mm³/10⁶ cells/h and the oxygen consumption 1.5 ± 0.2 mm³/10⁶ cells/h; RQ thus equalled 9.1. The contribution (negative) of the respiratory metabolism to the movement of the diver was in the order of 5 per cent, thus rather insignificant.

Figs. 5 and 6 demonstrate the migration of seven divers as measured over consecutive 10 min intervals. Fig. 5 shows the result of one experiment with exponentially multiplying cells (curve 1) and one with selection synchronized cells (curve 2). At time zero the small cells, separated from a culture in exponential growth by the gradient procedure of MITCHISON and VINCENT (15), were inoculated into fresh growth medium so as to give rise to a small synchronously multiplying culture. An exponentially (randomly) multiplying culture was prepared the same way except that the layers of the gradient used for the separation produce were mixed prior to the inoculation of the cell sample. It is seen that the glycolytic activity of the exponentially multiplying culture increases exponentially (curve 1) whereas the glycolytic activity of the selection synchronized culture

(curve 2) increases linearly between successive synchronous divisions. During the division periods the rate of linear increase about doubles as indicated by the values shown above the curve.

Fig. 6 shows the results of 5 experiments with heat synchronized cultures. Time zero denotes the end of the heat treatment (EH). The cells were transferred to the divers in the 32°C interval which precedes a final heat shock and thus received this shock while in the divers. As observed for selection synchronized cultures, the glycolytic activity increases linearly between consecutive synchronous divisions, and during each division the rate of linear increase about doubles as indicated by the values shown above each curve. Measured over several division cycles the overall increase in glycolytic rate is exponential.

Fig. 7 shows the results of Figs. 5 and 6 plotted on logarithmic ordinates. The straight dashed lines are fitted to the points by eye so as to indicate the general exponential increase of all the curves. Only the points from the experiment with exponentially growing cells (curve 1) actually fit a straight line. Curves 2-7 are wavy and this would be expected if there is arithmetic linear increase in fermentation rate from division to division as postulated above.

The values shown above each of the curves in Fig. 7 indicate the time (min) which it takes

Figure 5. The glycolytic activity of *S. pombe*. An exponentially growing culture (curve 1) and a culture synchronized by selection (curve 2). The two curves show upward migration rates of 2 divers ($\text{CO}_2 \cdot \text{t}^{-1}$). The frames delimit intervals with doubling of the cell number, between these the cell number was constant. The values shown above curve 2 indicate the slope of the straight lines. The divers were charged with about 200 cells (diver 1) and 300 cells (diver 2). The gas volume of the divers was about 1 mm^3 and the volume of growth medium about 0.1 mm^3 .

Figure 6. The glycolytic activity of *S. pombe*. Five cultures synchronized by heat shocks. The heat treatment was terminated at time zero (EH). For further explanation, see text to Fig. 5. The divers were charged with about 200 cells (diver 3) and 300 cells (divers 4 - 7). The gas volume of the divers was about 1 mm^3 and the volume of growth medium about 0.1 mm^3 .

mm / 10 MINUTES

(3)

(4)

(7)

(6)

(5)

(4)

(3)

(6)

(7)

EH

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1.6

2.0

1.8

2.1

1.8

1

1

1

1

1

for the glycolytic rate to double. This doubling time ranges from 115 to 130 min which is slightly less than the generation time (140 min) of cells multiplying exponentially in this medium and definitely more than the generation time of free running heat synchronized cells (95 min).

4. DISCUSSION

The present work shows that division synchrony in *S. pombe* in a chemically defined medium can be induced by heat shocks spaced

one generation time apart. As long as the cyclic heat shock program continues one synchronous division takes place during each of the low temperature periods (restricted synchrony). This is true after 4 to 6 shocks as shown in Fig. 8. After termination of the heat shocks two synchronous divisions follow (free running synchrony). In this work we have dealt only with free running synchrony.

The glycolytic activity increases linearly through the cell cycle of heat- and selection synchronized cells growing in EMM 2, in agreement with results obtained for cells in YEG (5).

Figure 7. The experiments of Figs. 5 and 6 shown with logarithmic ordinate. The time it takes for a doubling of the migration rate is indicated at the end of each curve.

Figure 8. Restricted synchrony. The effect of a series of heat shocks on cell multiplication. Upper frame: cells·ml⁻¹ versus time. Lower frame: variation with time in the fraction of cells with cell plates. The heat shocks are indicated by the numbered boxes. In this case each shock lasted 45 min.

These results differ from the observations of CREANOR (2) who found constant rate of CO₂ production between successive divisions with a sharp rate doubling at a specific time point in the interdivision period. The reason for this discrepancy is not clear to us but may be attributed to differences in methods and/or in strains.

The only fermentable substrate in EMM 2 is glucose. During the cell cycle a linear increase

in the rate of degradation of glucose is thus taking place. KUBITSCHKE and CLAYMAN (10) studied the uptake of glucose and glycine in synchronous cultures of *S. pombe* and found that both substrates are taken up at constant rate during the cell cycle. It is known that more than 90 per cent of the glucose which is taken up in yeasts is catabolized, whereas less than 10 per cent is assimilated (18). Taken together these observations suggest increase of the

glucose pool during the first half of the cell cycle, and decrease during the second half.

In EMM 2 the cell divisions are accelerated during free running synchrony (Table I), and they run at the same rate as in YEG. This is so in spite of faster exponential growth rate in YEG (5, 7). Nuclear division, DNA synthesis, cell plate formation and cell division are spaced similarly in heat and selection synchronized cultures. Consequently, the observed shortening of the free running heat synchronized cycle reflects compression of the early part of the cell cycle, and this compression differs in EMM 2 and YEG. The latter part of the cell cycle exhibits the sequence of nuclear division, DNA synthesis, cell plate formation and cell division, and this sequence displays itself in standard time, in normal and synchronized cells, in complex and in chemically defined medium. While the early part of the cell cycle is rather empty of demonstrable events (8, 12), growth occurs during this phase. Heat shock synchronized cells are about 40% oversized in YEG (7). The size of heat synchronized cells in EMM 2 was not measured, but visual observations indicate that also these cells are oversized compared to exponentially multiplying cells. It is the duration and extent of growth during the early part of the cell cycle which suffer reduction when the oversized cells again reduce towards normal size in the course of a few free running synchronized cell cycles. This reduction in cell size is also reflected at the level of fermentation rate as measured in YEG (5) and in EMM 2: cells in free running synchrony show doubling times for fermentation rate which considerably exceed the doubling time for cell numbers.

In conclusion, induction of synchrony by heat in EMM 2 results in a nearly normal cell cycle. This is in agreement with previous observations with cells growing in YEG (7).

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