

A CONTROL ACTING OVER THE INITIATION OF DNA REPLICATION IN THE YEAST *SCHIZOSACCHAROMYCES POMBE*

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SUMMARY

The control of cell division in the yeast *Schizosaccharomyces pombe* appears to be quite different to that of any other eukaryotic organisms for it is usually exerted not at the initiation of *S*-phase but at that of mitosis. However, it has been suggested that a control over the initiation of *S*-phase does also exist but that its action is redundant whilst the mitotic control is operating. This study has chosen conditions in which the latter appears to be largely absent in order to study the cryptic *S*-phase control. The timing of *S*-phase has been studied in cells grown at varying rates under nitrogen limitation in a chemostat. It is found that under these conditions the control of cell division resembles that of other eukaryotes. As the dilution rate of the chemostat is reduced, all increase in the generation time can be accounted for by a lengthened *G*₁ period. In contrast, the length of *S* + *G*₂ remains invariant. Thus, there must indeed be a control acting in *G*₁ in *S. pombe*. An analysis of the size of cells at different growth rates shows that the initiation of *S*-phase is correlated with a particular cell size.

INTRODUCTION

It is now widely believed that the control of cell proliferation in most eukaryotic organisms is exerted exclusively during the *G*₁ phase of the cell cycle (Prescott, 1976). The situation in the fission yeast *Schizosaccharomyces pombe* is, however, more complex. The isolation and analysis of *wee* mutants (Nurse, 1975) has shown that there is a control acting over mitosis. For instance, a significant proportion of the *G*₂ nuclei of its *wee* mutants, when shifted from a temperature at which the cells are the same size as wild type to one at which they are half the size, are immediately accelerated into mitosis.

This effect is also noted when wild type cells are shifted from one nutrient medium to another at which their steady-state size is smaller (Fantes & Nurse, 1977). Therefore, it appears that the major control over cell division in *S. pombe* lies not at the beginning of the cycle in *G*₁ but at the end just prior to mitosis. DNA replication is causally dependent upon previous mitosis in *S. pombe* (Nurse, Thuriaux & Nasmyth, 1976) and follows soon after it (Nasmyth, Nurse & Fraser, in preparation).

However, a further analysis of *wee* mutants has revealed that there is also some control, involving cell size, acting over the initiation of DNA replication (Nurse & Thuriaux, 1977). It is argued that this control is cryptic in wild type because the size

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of wild type cells at *S*-phase, which is directly determined by the control over mitosis in the previous cell cycle, is always above the critical size that a cell needs to attain in order to pass through the control acting over the initiation of DNA synthesis. The timing of *S*-phase in wild type cells is therefore determined by mitosis; that is, it occurs as soon after mitosis as is possible. In *wee* mutants where the size control acting over mitosis is abolished, the mean cell size is nearly halved and the cryptic size control acting over DNA replication is revealed. As a result, all *wee* mutants have a significant G_1 period of 0.3 of a cycle during which, it is argued, they are growing to a size critical for DNA replication to proceed.

This hypothesis has been tested by an analysis of the timing of *S*-phase under conditions where wild type cells are abnormally small (and incidentally the same size as *wee* mutants) and hence liable to reveal their control over the initiation of DNA replication (Nurse & Thuriaux, 1977). These conditions are the outgrowth of spores and of nitrogen-starved cells. Both these populations are initially arrested in G_1 . On reinoculation, both wild type and *wee* cells have a significant G_1 before *S*-phase occurs at the same cell size in both strains (6–8 pg protein/cell); a size that is comparable to the size at which *wee* cells growing in steady state undergo DNA synthesis. *wee* cells then have a short G_2 , whereas wild type cells have a long G_2 and eventually divide at twice the size. Thus, the G_1 observed in *wee* cells growing in minimal medium at steady state is not due to some *S*-phase delay directly caused by their altered genotype, but simply because they are smaller, and thereby the size control over the initiation of DNA synthesis is revealed.

The experimental evidence presented here concerns the existence of the postulated G_1 control in wild type cells. At present, the most significant delay in the initiation of *S*-phase, and hence evidence that it is held back by some control, is the 0.3 of a cell cycle delay in *wee* strains. The G_1 observed in wild type cells on reinoculation of spores or nitrogen-starved stationary cultures is not long and is subject to alternative interpretations since these cells are not in steady state. For instance, it is possible that the entire DNA-synthetic machinery of the cell is essentially 'closed down' in spores and nitrogen-starved cells and that the observed G_1 on reinoculation is simply due to the time taken to prepare for a round of DNA synthesis. Independent evidence is therefore required.

This paper describes the timing of *S*-phase in wild type cells growing at different rates under nitrogen limitation in a chemostat. The results show that under these conditions the initiation of *S*-phase is controlled by an event in G_1 which may monitor cell size or some other parameter correlated with cell size.

MATERIALS AND METHODS

Media and growth conditions

Batch cultures were grown at 25 and at 36 °C in a minimal medium consisting of (per l.): glucose, 20 g; ammonium sulphate, 5 g; magnesium chloride, 1 g; potassium hydrogen phthalate, 3 g; disodium hydrogen orthophosphate, 1.8 g; and traces and vitamins as described by Mitchison (1970). For complete medium, yeast extract (Difco) was added at a final concentration of 0.5 %.

The above minimal medium is limiting in glucose. For a medium limiting in nitrogen, $(\text{NH}_4)_2\text{SO}_4$ was omitted, Na_2SO_4 added to 1 g/l, and NH_4Cl to 30 mg/l. The final cell density obtained in this medium is 10 times lower than that obtained if NH_4Cl is no longer limiting.

Cell number and protein determination

Cell number was determined as described by Mitchison (1970). Protein determinations (except chemostat samples) and nuclear staining were performed as described by Nurse *et al.* (1976).

The protein content of cells growing in the chemostat was performed as follows. A 1.5-ml sample of the chemostat culture was immediately frozen and stored at -20°C . After thawing the cells were spun down at 14000 g, washed with distilled water and suspended in 0.3 ml of distilled water. Two 0.1-ml samples were hydrolysed in 1 M NaOH containing 2% sodium deoxycholate for 18 h at 32°C and then assayed for protein by the method of Lowry, Rosebrough, Farr & Randall (1951). Cell number was determined from a parallel 0.1-ml sample.

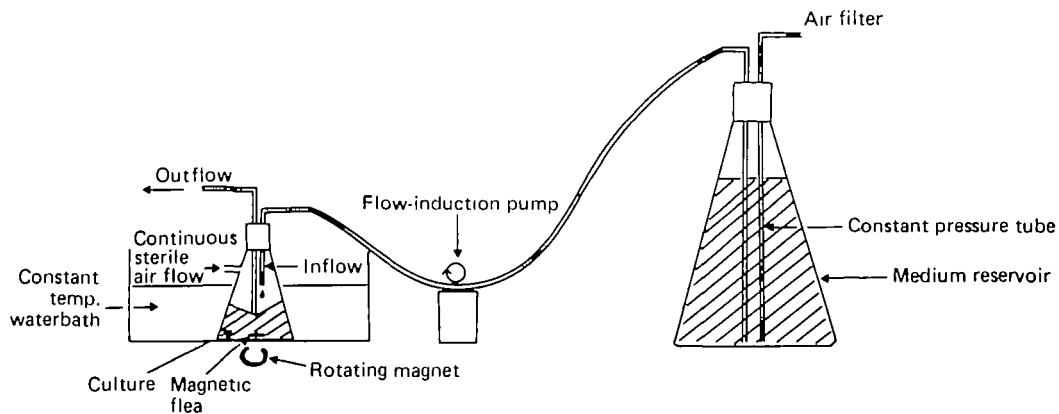


Fig. 1. The chemostat design.

Chemostat design

The chemostat design is shown in Fig. 1. The design was originally conceived by J. May. The whole chemostat was sterilized by autoclaving. The flow rate was varied by altering the rate at which fresh medium was pumped into the culture vessel. The latter was stirred continuously with a rotating magnet, thereby creating a vortex. Sampling was undertaken either by simply collecting the effluent or by stopping the stirrer, which causes collapse of the vortex and the consequent exudation of about 20 ml of culture medium.

Autoradiography of cells

Autoradiography of cells was performed as described by Nasmyth, Nurse & Fraser (in preparation).

RESULTS

The timing of S-phase in cells growing under nitrogen limitation in a chemostat

As has already been mentioned, the proposed reason why the hypothetical size control over the initiation of DNA synthesis is not revealed in 972 h⁻¹ growing in minimal medium is that the size control acting over the initiation of mitosis is set so high that cells, when they are ready to enter S-phase after mitosis, are always large enough to pass this control immediately. In order to observe this cryptic control

operating in wild type cells, it is necessary to find situations where cells are small enough to be controlled by it.

Nitrogen starved cells are predominantly arrested in G_1 (Nurse & Thuriaux, 1977; Egel & Egel-Mitani, 1974). Moreover, the cells are very small (3.9 pg protein/cell, see Table 2, p. 166). Therefore, the size control over mitosis must be inoperative when cells run out of ammonium ions in order that they can complete the mitotic cycle in the absence of growth and thereby arrest in G_1 . The control over mitosis may be either directly sensitive to the concentration of ammonium ions in the medium or only to some other internal metabolic state induced by its absence. Thus, it is possible that cells growing in a chemostat under ammonium limitation will also be free of the nuclear division size control due to the low concentration of ammonium in the medium under such limiting conditions.

The position of *S*-phase in cells growing in the chemostat has been analysed by 2 different methods. The first involves growing the *ts* mutant *cdc 10-129* in the chemostat at the permissive temperature (25 °C). *cdc 10-129* is a mutant with a temperature-sensitive defect in the initiation of DNA replication (Nasmyth, 1977). Therefore, if a sample of cells growing at the permissive temperature (25 °C) in the chemostat is withdrawn and immediately inoculated into fresh minimal medium at the restrictive temperature (36 °C), then the subsequent pattern of cell division should reveal the proportion of the asynchronous chemostat population that has completed the *cdc 10* initiation function, from which the *cdc 10* execution point (Hartwell, Culotti & Reid, 1970) may be calculated. There are several *ts* mutant alleles of *cdc 10* and all their execution points are mutually consistent (Nasmyth, 1977), so that it is likely that the execution point of *cdc 10-129* characterizes the completion of the function encoded by this gene rather than the thermosensitive decay of the mutant gene product. *cdc 10-129* reverts at a very low frequency and it is indefinitely stable in the chemostat at 25 °C. This method will of course only reveal the timing of an unknown *S*-phase initiation event, but it will also serve as the earliest estimate for the beginning of *S*-phase. The position of *S*-phase itself has been determined by autoradiography of cell DNA labelling *in situ*.

Fig. 2 shows the patterns of cell division, on reinoculation at 36 °C, of cells of *cdc 10-129* grown in the chemostat at a variety of different dilution rates. Fig. 3 shows the pattern of protein synthesis of a reinoculated culture. The first fact that emerges from these experiments is that cells grown in the chemostat are very much smaller than those grown in batch culture in high concentrations of ammonium ions. For instance, cells growing with a generation time of 13-14 h in the chemostat have a mean protein content of 5.2 pg/cell as opposed to the normal 12.5 pg/cell (data from Fig. 3). When ammonium ions are restored in high concentration as the cells are shifted from the chemostat to fresh minimal medium, the nutrient-modulated size control acting over the initiation of mitosis (Fantès & Nurse, 1977) is restored and mitosis is inhibited until the cells have grown large enough to pass this size control; hence the long first plateau in the cell number curves of Figs. 2 and 3. The cells resume division only when the normal cell size for these conditions (15-16 pg protein/cell) has been attained. The final plateau in the cell number curves is the result of the block imposed by the

ts mutation. Wild type controls continue exponentially at this point. The inhibition of mitosis on shift up is exactly analogous to that observed when *S. pombe* is shifted from a slow-growing medium with proline as nitrogen source to a fast one with ammonium as nitrogen source (Fantès & Nurse, 1977).

It is apparent that the size of cells grown in the chemostat should be small enough to reveal the hypothetical size control acting on the initiation of DNA synthesis. It

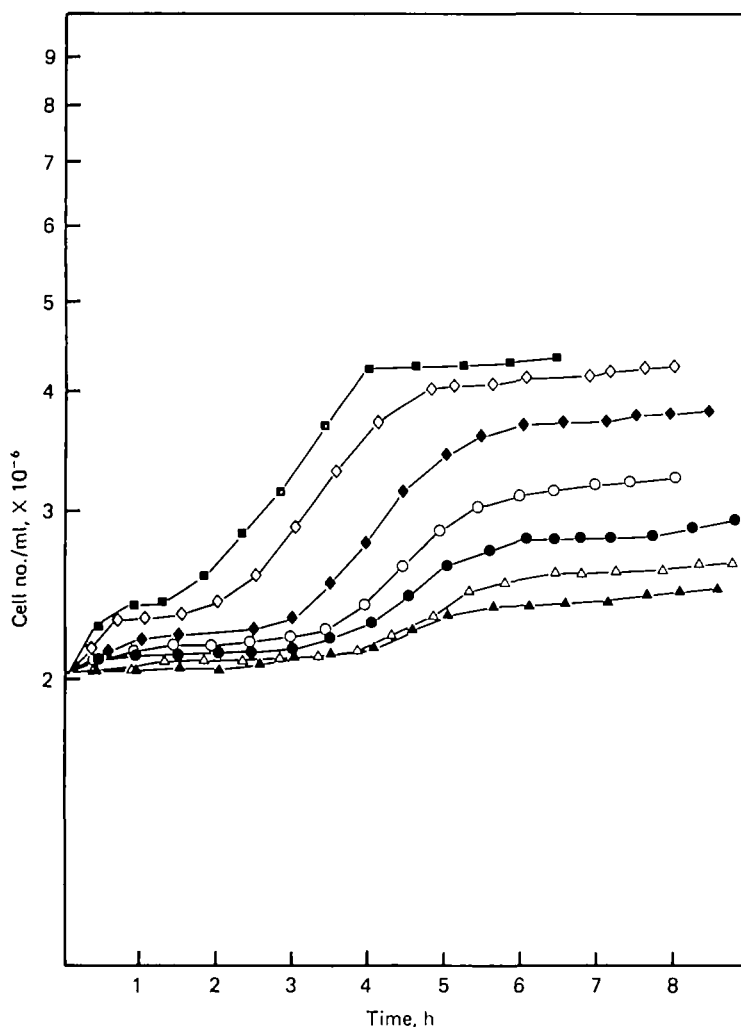


Fig. 2. The pattern of cell division when cells of *cdc 10-129 h⁻*, growing at 25 °C in the chemostat at various dilution rates, are inoculated into fresh minimal medium at 36 °C. The diagram is a composite of many different shift up experiments. Each cell number per ml at $t = 0$ has been aligned at the same point. The actual cell number per ml for each experiment was approximately as shown. ▲, 0.0376 h⁻¹; △, 0.0526 h⁻¹; ●, 0.0792 h⁻¹; ○, 0.1045 h⁻¹; ◆, 0.1314 h⁻¹; ◇, 0.1528 h⁻¹; ■, the pattern of cell division of *cdc 10-129 h⁻* when shifted from 25 to 36 °C in batch minimal medium (0.1733 h⁻¹). The short plateau in the cell number curve of the latter after 1 h at 36 °C has been discussed by Nurse *et al.* (1976).

appears that the size control over mitosis is mostly, if not totally, abolished under the conditions of growth in the chemostat. Table 1 contains a summary of *cdc 10* execution points for cells grown over a wide range of growth rates, as calculated from the cell division data of Fig. 2. It is clear that the position of the *cdc 10* execution point

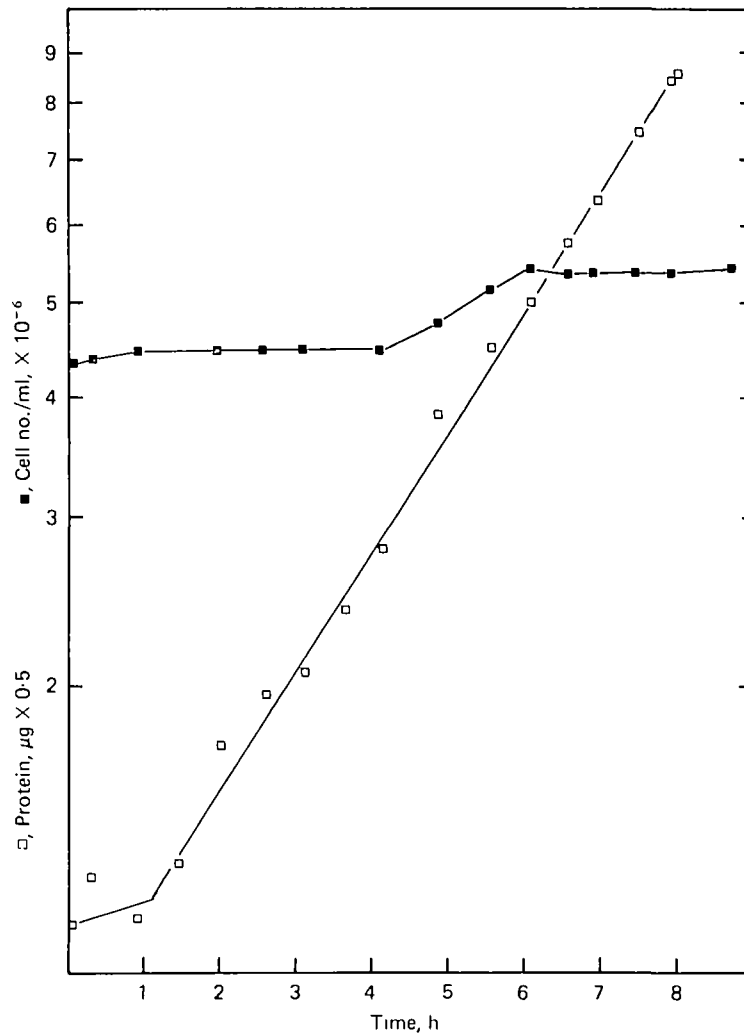


Fig. 3. The pattern of protein synthesis of *cdc 10-129 h⁻* cells when shifted from the chemostat (0.05 h^{-1} dilution rate) to fresh minimal medium at 36°C . Protein was determined from a 5-ml sample of the culture collected on a filter. These cells were suspended in 1 ml of *d.H₂O*, 0.1 ml of which was hydrolysed in alkali (see Methods) and assayed by the method of Lowry *et al.* (1951). At the beginning of the experiment, the mean protein content per cell was 5.18 µg. At the mid point of the transient cell number increase, the mean value was 15.7 µg/cell.

changes by more than a cell cycle over a 6-fold variation in the growth rate. At very fast growth rates (e.g. a 3-h mgt, as in complete medium), the *cdc 10* gene product appears to complete its function immediately after the mitosis in the previous cell

cycle, whereas at very slow growth rates (e.g. an 18-h mgt in the chemostat) it does not complete its function until 0.18 of a cell cycle prior to the subsequent mitosis. Over this range of growth rates the position of mitosis only varies from 0.7 to 0.9-0.95 in the cell cycle. Table 1 also shows the range of calculated times from the *cdc 10* execution point to cell division. This parameter, which will be a maximum estimate of the length of $S+G_2$, remains remarkably constant over the whole range of growth

Table 1. *The timing of the cdc 10 execution point at different growth rates*

<i>mgt</i> , h	Medium	Proportion of <i>cdc 10-129 h⁻</i> cells to divide on shift	<i>cdc 10</i> execution point	Time from execution point to cell division, h	Position of mitosis
∞	Stationary-phase cells grown in chemostat medium	0.00	+1.0	—	—
18.43	Chemostat	0.168	+0.776	4.13	—
13.80	Chemostat	0.230	+0.70	4.14	—
13.18	Chemostat	0.271	+0.654	4.56	—
10.43	Chemostat	0.320	+0.600	4.17	0.905
8.75	Chemostat	0.401	+0.513	4.26	—
6.63	Chemostat	0.571	+0.348	4.32	—
5.28	Chemostat	0.858	+0.106	4.72	—
5.26	Chemostat	0.780	+0.170	4.36	0.78
4.54	Chemostat	1.060	-0.045	4.74	—
4.00	Batch minimal medium	1.14	-0.100	4.40	0.795
3.07	Batch complete medium	1.48	-0.310	4.02	0.683

cdc 10-129 h⁻ was grown at 25 °C and its execution point was calculated from the proportion of cells that divided when the culture was shifted to 36 °C. In the case of batch cultures, culture flasks were simply shifted from a 25 °C waterbath to a 36 °C waterbath. For chemostat samples, the cells were inoculated into fresh minimal medium at 36 °C. The position of mitosis was calculated by the number of binucleate cells in the population.

rates. Therefore, it is clear that the length of $S+G_2$ is almost independent of the growth rate under these circumstances. All the variation in cell cycle time is due to a variation in the length of G_1 . The data thus corroborate the hypothesis of Nurse & Thuriaux (1977) that *S. pombe* not only has a control over the initiation of mitosis but also has an event in G_1 which controls entry into S -phase.

The data on cell division in *cdc 10-129* on shift up out of the chemostat only reveal the proportion of cells that have completed the *cdc 10* function. However, it is important to establish whether the completion of the *cdc 10* function is dependent upon the position of the cell in the cell cycle. This has been investigated by an analysis of cell division in *cdc 10-129* on shift up out of the chemostat by time-lapse photomicroscopy (see Fig. 4). Cells below a certain size, as indicated by their cell length at the time of reinoculation, have not completed the *cdc 10* function, whereas those above

this size, in general, have. In this same experiment the rate of growth of cells through the cell cycle in the chemostat has been investigated by autoradiography of cells pulse labelled with [^3H]leucine. This shows that the rate of protein synthesis increases continuously with cell size (see Fig. 4). The data are consistent with an exponential growth of cells as they progress through the cycle. Thus, it appears that even the G_1 cells of chemostat populations are growing and that cells do not complete the *cdc 10* function until they attain a certain cell size.

It is now important to establish the actual position of *S*-phase (the present estimates

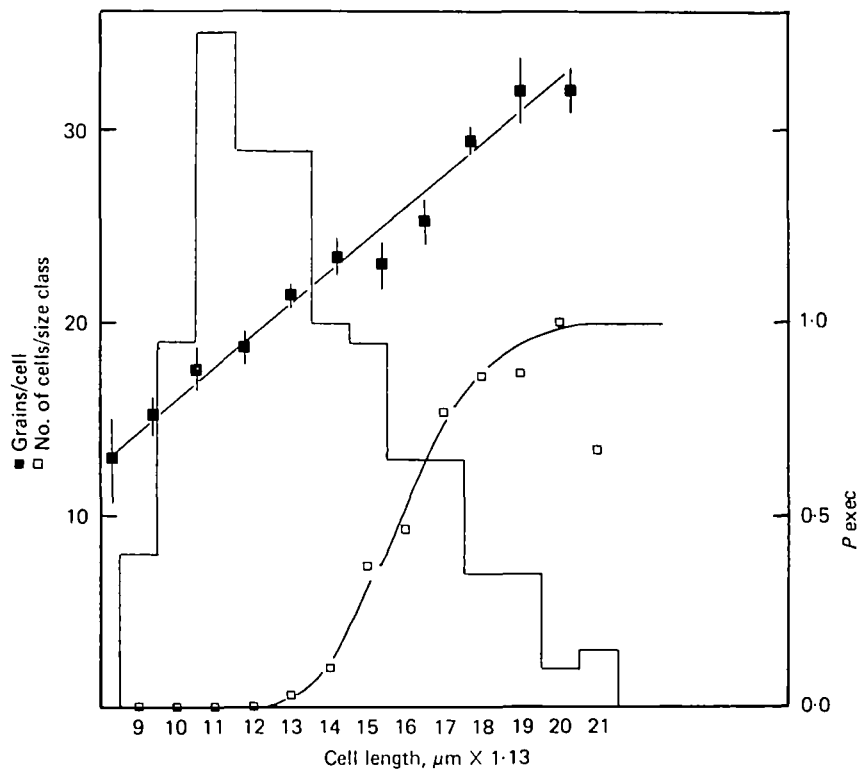


Fig. 4. The rate of protein synthesis and the proportion of cells that have executed the *cdc 10* function amongst cells of different sizes in the chemostat. Cells of *cdc 10-129 h⁻¹* were grown in the chemostat at 25 °C at a dilution rate of 0.0615 h⁻¹. The experiment consisted of two parts. (1) A sample of chemostat cells was removed and placed in a constant temperature growth chamber (minimal medium, 36 °C) on a microscope slide as described by Fantes (1977). The resulting pattern of cell division of these cells was then analysed by time-lapse photomicroscopy (Fantes, 1977). Thus, it was possible to determine which cells in the sample had executed the *cdc 10* function at the time of the shift by observing whether individual cells divided once or not at all at 36 °C. □ represents the proportion of a size class that had executed the *cdc 10* function at the time of sampling (*P. exec*). (2) The same chemostat culture was pulse labelled for 1 h with 4 μCi/ml [^3H]leucine (54 Ci/mM) while growing in steady state. The cells were then washed in cold 10% TCA and autoradiographed as described in Methods. ■ represents the number of grains per cell and its standard error for each size class. The size distribution of the population is also presented as a histogram so that the relation between cell length and cell cycle progress can be properly evaluated.

of the length of G_1 and G_2 are entirely based on the execution point of a *ts* mutant defective in some, as yet, unknown aspect of initiation of S -phase). To do this, a sample of $972 h^-$ cells growing in the chemostat at a 9-h mgt was pulse labelled with [$6\text{-}^3\text{H}$]uracil, fixed, and treated for DNA specific autoradiography as described by Nasmyth *et al.* (in preparation). Fig. 5 shows the size distribution of cells with labelled nuclei. It is clear that the distribution of labelled nuclei amongst cells of different size classes is very different to that found when batch-grown cells are pulse labelled. There, the nuclei of cells with a cell plate are almost exclusively labelled (Fig. 6). Here, only

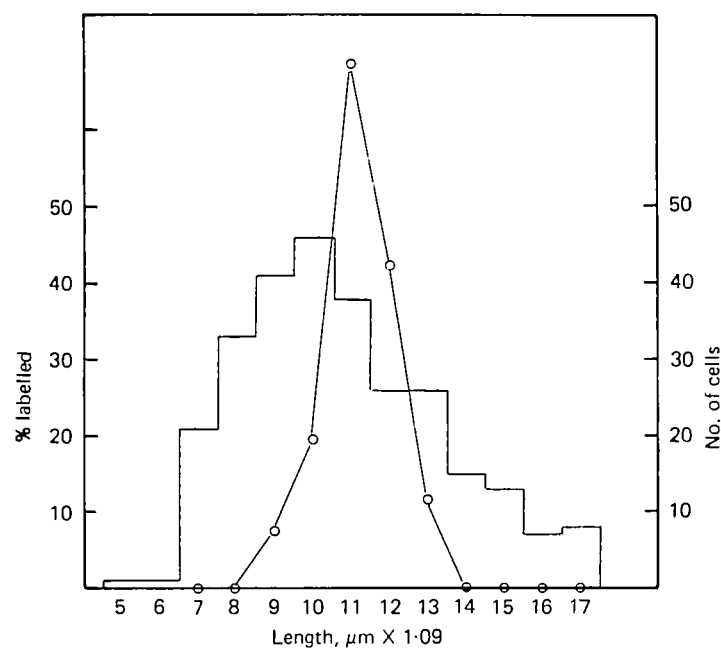


Fig. 5. The position of S -phase in cells growing in the chemostat. $972 h^-$ cells were grown in the chemostat at $0.0744 h^{-1}$ (25°C). A 2-ml sample was removed and incubated for 25 min in $50 \mu\text{Ci/ml}$ [$6\text{-}^3\text{H}$]uracil. These cells were treated for DNA-specific autoradiography as described by Nasmyth *et al.* (in preparation). The percentage of cells with labelled nuclei is plotted for each size class. The size distribution of the culture is also presented. The mid point of S -phase (with respect to time) is calculated as follows. The number of cells with labelled nuclei are cumulatively counted, starting with the smallest and proceeding through successively larger size classes. After correction for the age distribution (Cook & James, 1964), the proportion of the cell population (on the small size) within which are half the labelled cells, gives the cell cycle position of the mid point of S -phase.

nuclei from cells of intermediate cell length are labelled (Fig. 5). The position of the mid-point of S -phase is estimated to be at 0.53 in the cell cycle (the details of this calculation are described in the legend of Fig. 5). This position is very close to the execution point of *cdc 10-129* under these conditions. Here too, the position of S -phase is associated with a particular cell size; cells both smaller and larger than this size are unlabelled (Fig. 5).

The size of cells growing in the chemostat

The hypothesis that there is a critical size for the initiation of DNA replication and a constant $S+G_2$ period makes precise predictions concerning the size of cells at different growth rates. Nurse & Thuriaux (1977) considered the protein content of cells. This convention is maintained here.

Let: S_d = the protein content of the cell at cell division; S_l = the protein content

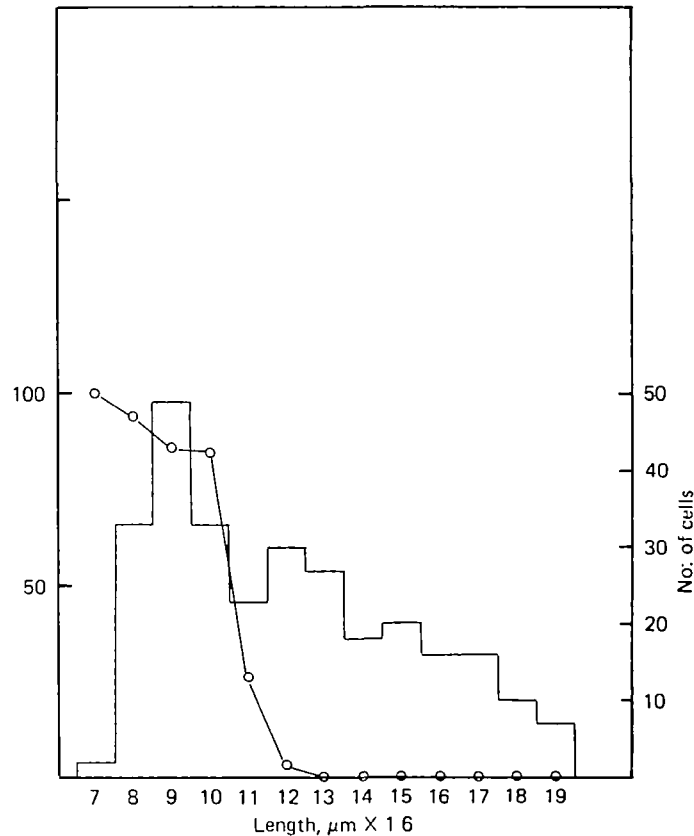


Fig. 6. The position of S -phase in cells growing in minimal medium. $972 h^-$ cells growing at $32^\circ C$ in minimal medium were pulse labelled with $[^3H]$ guanosine for 20 min and treated for DNA specific autoradiography as described by Nasmyth *et al.* (in preparation). \circ represents the % of cells in a size class that are labelled. During the scoring, cell-plate cells were regarded as 2 independent daughter cells. The size distribution of the sample of cells scored is also presented as a histogram so that the relation between cell length and stage in the cell cycle can be properly interpreted.

of the cell at the initiation of DNA synthesis; S_m = the mean cell protein content; C = the constant period between the initiation event and cell division; mgt = the mean generation time of the culture; and d = the growth rate of the culture. Now the autoradiographs of cells pulse labelled with $[^3H]$ leucine (Fig. 4) showed that the rate of protein synthesis increases continuously during the cell cycle of cells

grown in the chemostat. Therefore, we may assume that the protein content of cells increases exponentially through the cell cycle.

$$\text{Then: } S_d = S_1 \cdot 2^{(C/mt)}$$

From which it follows that: $\log_e(S_m/\log_e 2) = \log_e S_1 + C \cdot d$. Therefore, the precise predictions of the hypothesis are: (1) The log of the mean protein content of cells should be directly proportional to the growth rate d . And (2) If $\log_e(S_m/\log_e 2)$ is plotted against d , then the intercept should be $\log_e S_1$ and the slope should be C . That is, the intercept reveals the protein content at initiation and the slope reveals the constant period between the initiation event and cell division.

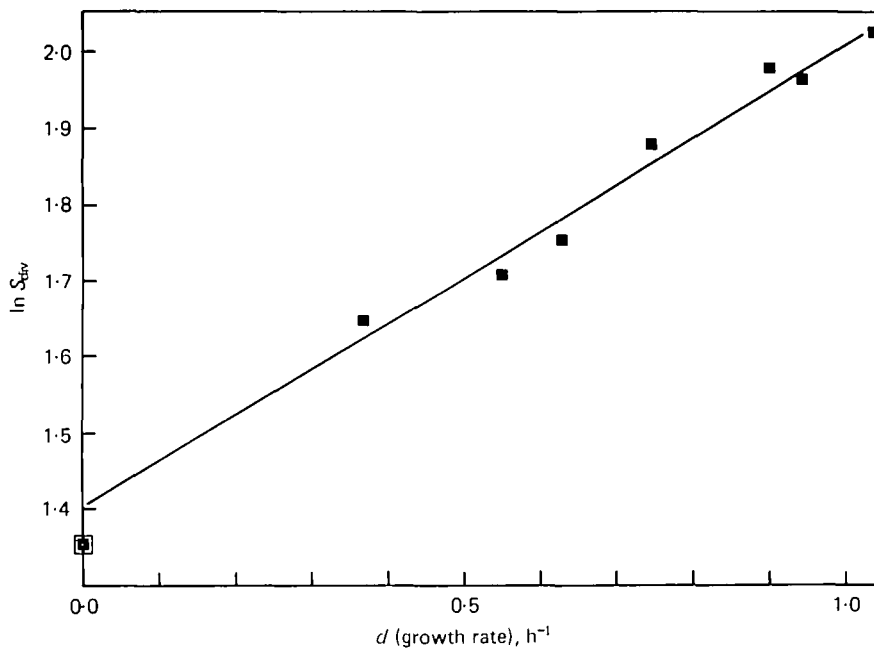


Fig. 7. A plot of $\log_e S_d$ (the protein content of cells at division) against d (the relative growth rate). 972 h^{-1} cells were grown at 25°C at different dilution rates. The mean protein content per cell (S_m) was determined as described in Methods. It was assumed that $S_d = S_m/\log_e 2$. The line drawn was calculated from a linear regression of the data. The point for $d = 0$ represents stationary phase cells which have been grown in medium of the same composition as that used in the chemostat. This point is not included in the linear regression.

Fig. 7 shows data on the protein content per cell of 972 h^{-1} at different growth rates plotted accordingly. The slope of the line gives an estimate for the constant period C of 6 h, and the intercept gives a value of 4.06 pg/cell . The latter, as expected, is close to the size of nitrogen-starved stationary-phase cells, which is $3.87 \text{ pg protein/cell}$ (Table 2). The fit of the data to the theoretical predictions can be evaluated in either one of two different ways.

First, the value of C derived from the slope of the line of Fig. 7 can be compared with the estimate of the constant period from the *cdc 10* execution point to cell division ($C_{cdc 10}$). The mean value of $C_{cdc 10}$ from 8 chemostat runs at different growth

rates (Table 1) is 4.42 h. As already mentioned, there is no significant variation in this period as the growth rate is varied. Therefore, the estimate of 6 h for C from the slope of the protein content per cell curve of Fig. 7 is probably, despite the scatter in the data, significantly different from the value of 4.42 h calculated for C_{cdc10} . This difference implies that cell size, as determined by protein content per cell, is not perfectly correlated with the execution of the G_1 event controlling entry into S -phase. The control may be operated by some other factor than protein content *per se*. The latter may be only correlated to a certain extent with the former. Alternatively, it is possible that the hypothetical G_1 size control in fact executes (under the chemostat growth conditions) 1.6 h prior to the $cdc 10$ execution point. If this were true, then the latter would not be strictly correlated with a particular cell size.

Table 2. *The protein content of cells at the cdc 10 execution point ($S_{cdc 10}$) at different growth rates*

d, h^{-1}	$S_d, \text{pg/cell}$ (actual)	$S_d, \text{pg/cell}$ (regression)	$cdc 10$ execution point	$S_{cdc 10},$ pg/cell
0.00	3.87	4.06	1.00	4.06
0.0366	5.20	5.07	0.7666	4.313
0.0548	5.525	5.654	0.6505	4.438
0.0628	5.77	5.934	0.5995	4.498
0.0744	6.39	6.364	0.5256	4.581
0.0898	7.214	6.983	0.4273	4.695
0.0946	7.11	7.188	0.3967	4.733
0.10365	7.56	7.591	0.3390	4.801

972 h^{-1} cells were grown in the chemostat at 25 °C. 1.5-ml samples of the chemostat culture were collected from the outlet and stored at -20 °C. The mean protein content per cell (S_m) was measured as described in Methods. It was assumed that cells growing in the chemostat grow at an exponential rate through the cell cycle (see Fig. 4). Therefore, $S_d = S_m / \log_e 2$. S_{cdc10} was calculated by assuming (a) the value of S_d predicted from the linear regression of Fig. 7, and (b) that the $cdc 10$ execution point was always 4.42 h prior to division. (The data are presented in this way because actual estimates of the $cdc 10$ execution point were not always available for each particular growth rate for which there were data on the size of cells.)

A second, though not independent, way of analysing the results is to calculate the protein content per cell at the $cdc 10$ execution point throughout the range of growth rates. This is done in Table 2, where it is found that the predictions of the G_1 size control hypothesis are partially fulfilled. Whereas the position of the $cdc 10$ execution point and the mean protein content of cells vary considerably at different growth rates, the protein content of cells at the time of the $cdc 10$ execution point remains fairly constant (4.3–4.8 pg protein/cell). Moreover, this protein content is comparable to that predicted from the intercept of Fig. 7 (4.06 pg/cell).

Finally, it is of interest to compare the size at which cells initiate DNA synthesis in the chemostat with the critical cell size proposed by Nurse & Thuriaux (1977). The mean protein content of 972 h^{-1} cells grown in batch minimal medium is 13.6 pg/cell, if estimated by the method used for the analysis of cell size in chemostat samples. This is almost identical to estimates made by the method of Nurse &

Thuriaux (1977). Therefore, the protein contents reported here can be directly compared to theirs. They estimated that the mid-point of *S*-phase in spore outgrowth, in *wee* cells in batch culture, and in reinoculated nitrogen-starved cells occurred at 6–7 pg protein/cell. This compares to the range of 4.3 to 4.8 pg protein/cell for the size at which the *cdc 10* event is executed in cells grown in the chemostat. Part of this discrepancy can be accounted for by the time difference between the *cdc 10* execution point and the mid point of *S*-phase. Despite this, a significant difference still remains. However, it is perhaps not surprising that cells grown in the chemostat under NH_4^- limitation have lower protein contents under comparable control situations (i.e. in the absence of the control acting over the initiation of mitosis). As already mentioned, the factor responsible for triggering the execution of the G_1 control is unlikely to be the protein content of the cell *per se*. In which case, it is only fair to compare similar physiological situations.

A possible explanation of the discrepancy is that the G_1 control is modulated by nutrient conditions in a manner analogous to the mitotic control (Fantès & Nurse, 1977).

DISCUSSION

The results reported in this paper are simple. When cells of *S. pombe* are grown under nitrogen limitation in a chemostat, the length of $S + G_2$ remains constant despite great variation in the growth rate of the culture. A corollary of this is that all increase in the generation as the dilution rate is reduced can be accounted for by an increase in the length of G_1 . This result, which implies the existence of a control in G_1 regulating entry into *S*-phase, has of course been documented in countless other eukaryotic organisms (Prescott, 1976), and might therefore not appear to be very surprising. It is, however, of considerable interest in the particular case of *S. pombe*, for in this organism the existence of such a control has been in doubt due to it being obscured by an alternative control over the initiation of mitosis (Nurse & Thuriaux, 1977). At fast growth rates in rich media, the requirements of the mitotic control appear to be more rigorous than the G_1 control so that the former determines the size and time at which cells divide and the latter is largely redundant. However, under poorer nutrient conditions, as appear to pertain in a nitrogen-limiting chemostat, the mitotic control appears to be relaxed and the G_1 control is therefore engaged. *S. pombe* appears to be unique in having 2 different control points within the cell cycle.

Nurse & Thuriaux (1977) have suggested that the passage of a *S. pombe* cell through the G_1 control is dependent upon it reaching a critical size. This hypothesis has been broadly corroborated by the chemostat study. It was found that although both the mean cell size and the position of *S*-phase vary considerably when cells are grown at different rates in chemostat, the size at which cells initiate DNA replication remains relatively constant.

Evidence that the events immediately leading up to DNA replication are dependent on the cell reaching a critical size is to be found in several other eukaryotic organisms; for instance, in mouse fibroblasts (Killander & Zetterberg, 1965) and in the budding yeast *S. cerevisiae* (Johnston, Pringle & Hartwell, 1977).

REFERENCES

- COOK, J. R. & JAMES, T. W. (1964). Age distribution of cells in logarithmically growing cell populations. In *Synchrony in Cell Division and Growth* (ed. E. Zeuthen). New York: Inter Science Publishers.
- EGEL, R. & EGEL-MITANI, M. (1974). Premeitotic DNA synthesis in fission yeast. *Expl Cell Res.* **88**, 127-134.
- FANTES, P. A. & NURSE, P. (1977). Control of the cell size at division in fission yeast by a growth modulated size control over nuclear division. *Expl Cell Res.* **107**, 377-387.
- HARTWELL, L. H., CULOTTI, J. & REID, B. (1970). *Proc. natn. Acad. Sci. U.S.A.* **66**, 352.
- JOHNSTON, G. C., PRINGLE, J. R. & HARTWELL, L. H. (1977). Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. *Expl Cell Res.* **105**, 79-98.
- KILLANDER, D. & ZETTERBERG, A. (1965). Quantitative cytochemical studies on interphase growth. *Expl Cell Res.* **38**, 272-284.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein estimation with the Folin phenol reagent. *J. biol. Chem.* **193**, 265-275.
- MITCHISON, J. M. (1970). Physiological and cytological methods for *Schizosaccharomyces pombe*. In *Methods in Cell Biology*, vol. 4 (ed. D. M. Prescott), pp. 131-165. New York: Academic Press.
- NASMYTH, K. A. (1977). *DNA Replication in Schizosaccharomyces pombe*. PhD Thesis, Edinburgh University.
- NURSE, P. (1975). Genetic control of cell size at cell division in yeast. *Nature, Lond.* **256**, 547-551.
- NURSE, P., THURIAUX, P. & NASMYTH, K. (1976). Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. *Molec. gen. Genet.* **146**, 167-178.
- NURSE, P. & THURIAUX, P. (1977). Controls over the timing of DNA replication during the cell cycle of fission yeast. *Expl Cell Res.* **107**, 365-377.
- PRESCOTT, D. M. (1976). The cell cycle and control of cellular reproduction. *Adv. Genet.* **18**, 100-177.

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