UNEQUAL DIVISION IN *SACCHAROMYCES CEREVISIAE* AND ITS IMPLICATIONS FOR THE CONTROL OF CELL DIVISION

LELAND H. HARTWELL and MICHAEL W. UNGER

From the Department of Genetics, SK-50, University of Washington, Seattle, Washington 98195

ABSTRACT

The budding yeast, *Saccharomyces cerevisiae,* was grown exponentially at different rates in the presence of growth rate-limiting concentrations of a protein synthesis inhibitor, cycloheximide. The volumes of the parent cell and the bud were determined as were the intervals of the cell cycle devoted to the unbudded and budded periods. We found that *S. cerevisiae* cells divide unequally. The daughter cell (the cell produced at division by the bud of the previous cycle) is smaller and has a longer subsequent cell cycle than the parent cell which produced it. During the budded period most of the volume increase occurs in the bud and very little in the parent cell, while during the unbudded period both the daughter and the parent cell increase significantly in volume. The length of the budded interval of the cell cycle varies little as a function of population doubling time; the unbudded interval of the parent cell varies moderately; and the unbudded interval for the daughter cell varies greatly (in the latter case an increase of 100 min in population doubling time results in an increase of 124 min in the daughter cell's unbudded interval). All of the increase in the unbudded period occurs in that interval of G1 that precedes the point of cell cycle arrest by the *S. cerevisiae* α-mating factor. These results are qualitatively consistent with and support the model for the coordination of growth and division (Johnston, G. C., J. R. Pringle, and L. H. Hartwell. 1977. *Exp. Cell. Res.* **105:**79–98.) This model states that growth and not the events of the DNA division cycle are rate limiting for cellular proliferation and that the attainment of a critical cell size is a necessary prerequisite for the "start" event in the DNA-division cycle, the event that requires the *cdc* 28 gene product, is inhibited by mating factor and results in duplication of the spindle pole body.

KEY WORDS *Saccharomyces cerevisiae* · yeast · division kinetics · unequal division · G1 control · growth control

Observations made with organisms as diverse as bacteria, fungi, and animal cells suggest that the attainment of a critical cell mass is a necessary prerequisite for the initiation of the cell cycle, an event that is usually evidenced by the onset of DNA replication. In 1968 Donachie (7) noted that the observations of Schaechter et al. (27), demonstrating a proportionality between the log of the individual cell mass and the growth rate for *Salmonella typhimurium* taken together with the Cooper and Helmstetter (6) model for the timing of DNA replication in *Escherichia coli*, revealed that the initiation of chromosome replication took

place at integral multiples of a particular cell mass. Killander and Zetterberg (19) observed that mouse cells in culture had a smaller variation in mass and a larger variation in age at the onset of DNA synthesis than they exhibited at division "suggesting that the intiation of DNA synthesis is more related to the mass than to the age of the cell." Other observations suggesting a similar relationship between cell size and the onset of DNA synthesis have been made for Chinese hamster cells (20) and human lymphoid cells (36). However, Fox and Pardee (9) failed to find such a relationship in Chinese hamster ovary cells. A particularly enlightening example is provided by the yeast *Schizosaccharomyces pombe* where the control of DNA synthesis by cell size is cryptic under conditions of rapid growth but can be demonstrated upon nutritional deprivation (25).

S. cerevisiae permits a rather dramatic demonstration of the relationship between growth and division. During nutrient starvation parent cells produce extremely small daughters which result from the unequal distribution of mass between parent and bud, a situation not usually encountered in organisms that divide by binary fission. The interval of time from the addition of fresh nutrients to starved cells until the initiation of a new cell cycle is inversely related to the initial size of the cell because all cells grow to approximately the same size before initiating a new cell cycle (18). Other experiments demonstrate that cycles once initiated can be completed with little or no net growth, a result indicating that the growth requirement is unique for a particular step in the cell cycle.

The event in the *S. cerevisiae* cell cycle that is uniquely sensitive to cell size has been located at or before the step in the G1 interval of the cell cycle that is controlled by the product of gene *cdc* 28 (15). Expression of the *cdc* 28 product is essential for the duplication of the spindle pole body on the nuclear membrane (4). The *cdc* 28 mediated step precedes the actual initiation of DNA replication by at least two other steps, those controlled by the products of genes *cdc*4 and *cdc* 7 (15). The *cdc* 28 controlled step is also the step at which mating factors arrest haploid cells, apparently in order to synchronize the two cell cycles before cell fusion during conjugation (3, 34), and hence sensitivity to mating factor provides a convenient test for whether or not a particular cell has passed this point of control. Starvation of prototrophic *S. cerevisiae* cells for any one of a variety of essential nutrients also synchronizes the cell cycles at the *cdc* 28 step (2, 30, 35, Pringle and Maddox, personnal communication). The *cdc* 28 mediated step has been termed "start" because it controls the commitment of the cell to division (12).

The experiments that demonstrated a correlation between completion of the start event and the attainment of a critical cell size in *S. cerevisiae* involved shifting cells from nutrient-sufficient to nutrient-deficient conditions and vice versa as well as shifts of temperature-sensitive mutants to the restrictive temperature (18). It is possible that the change in conditions imposed upon the cell during these shifts induced control mechanisms that do not operate during steady-state growth. For example, the ability of the cell to divide before the daughter bud has attained a size comparable to that of the parent after nutrient starvation might be a special property of starved cells. It is the purpose of this report to examine the growth and division of *S. cerevisiae* cells under steady-state conditions to determine whether the hypothesis of a size requirement for completion of the start event remains tenable.

MATERIALS AND METHODS

Yeast Strains, Media, and Culture Conditions

Most of the experiments reported herein were done with a prototrophic a/α diploid strain, C276, whose origin was described previously (8, 34). A variety of strains including haploids, diploids, and temperature-sensitive mutants were utilized for the experiments of Fig. 6 as follows: DU-MES-1 (30), ts 341 (13), ts 187 (14), 2180A (34), met2a (30), and met 2 a/α.

For all experiments except a few of those reported in Fig. 6, cells grown in liquid medium were in YNB (18) and those grown on solid medium were on YNB containing 10 g/liter noble agar (Difco Laboratories, Detroit, Mich.). In a few experiments reported in Fig. 6, cells were grown in YNB containing supplements for auxotrophic requirements or in YM-1 (10).

Cells were grown on solid medium or in liquid medium in flasks with rotary shaking at a temperature of 22°–24°C. Cells grew more slowly in liquid medium than on solid medium despite low ratios of culture medium to flask volume and rapid shaking, and the cells in liquid displayed a higher proportion of unbudded cells. The difference in the fraction of unbudded cells was just about what would be produced by slowing down the growth rate with cycloheximide on solid medium to that observed without cycloheximide in liquid medium. Although it is not necessary to compare directly cells grown in liquid to those grown on solid medium for the argu-

ments to be made below, it is probably correct to compare the cells that are growing at the same growth rate under the two conditions rather than to compare cells growing with the same concentration of cycloheximide.

The viability of strain 2180A cells growing in a steady state in YNB liquid medium containing various concentrations of cycloheximide was determined. One thousand individual cells were scored by time-lapse photomicroscopy for their ability to form microcolonies on solid medium without cycloheximide. Over the range of concentrations of cycloheximide used in the experiments reported in this paper, between 93 and 99% of the cells were viable.

Measurement of Cell Parameters

Procedures for determination of the cell number, the proportion of unbudded cells (18), and the number of bud scars per cell (5) have been described previously.

The volumes of individual cells were calculated from phase-contrast micrographs, assuming that the yeast cell is a prolate spheroid (28). The micrographs were enlarged by projection and the major and minor axes of the cell were measured with a graf/pen digitizer (model GP-3) (Science Accessories Corp., Southport, Conn.) interfaced with a Hewlett-Packard calculator (model 9820A) (Hewlett-Packard Co., Palo Alto, Calif.). To determine the magnification so that absolute cell volumes could be obtained, the grid system of a Petroff-Hausser (C. A. Hausser & Son, Philadelphia, Pa.) counter was photographed with the same optical system, and the magnification was calculated from repeated measurements of this standard. Some cell volume distributions were also obtained with a particle size distribution analyser (Coulter Channelyzer, Coulter Electronics Inc., Hialeah, Fla.). The analyzer was calibrated using 22.26- and 73.62-μm^3 polystyrene beads.

Time-Lapse Photomicroscopy

An overnight stock culture grown in YM-1 medium was diluted 10- or 30-fold and 0.1 ml was spread onto a YNB-agar plate. Cells were pregrown for 18–24 h at room temperature (22°–24°C) on plates containing 1% noble agar (Difco Laboratories) and the same concentration of nutrients and inhibitor to be used in the time-lapse photography. Cells were washed off of the plate with 1 ml of YNB liquid medium, agitated on a vortex mixer for 30 s, and a drop was placed onto a 12 × 30 × 1 mm slab of agar. The cells were allowed to settle out for 1–2 min, and then the slide was placed in a vertical position to permit the liquid to run off the cells and the surface to dry. A nylon screen (1.5 mm between fibers) that had been previously washed in ethanol and water was placed over the cells to provide a frame of reference. Photographs were taken at room temperature (22°–24°C) at intervals of 10–20 min for 6–12 h depending upon the growth rate of the cells. Individual cells were then scored for their pattern of budding from the projected negatives. All initially unbudded cells were scored until a total of five cell units (a unit is a parent cell or a bud) had appeared, and all scored cells are reported unless they could not be followed unambiguously (due to crowding) for the full course of the experiment. When cells were pregrown in liquid medium or sonicated before the time-lapse experiments, then the population exhibited deviations from exponential growth during time-lapse photography and hence these procedures were not used.

RESULTS

Unequal Division

Exponentially growing populations of *S. cerevisiae* C276 were followed by time-lapase photography. The doubling time of each population was determined by counting the total number of cell units (each parent cell and each bud is a unit) in the same field at successive times (Fig. 1). Strain C276 exhibited exponential growth on YNB-agar

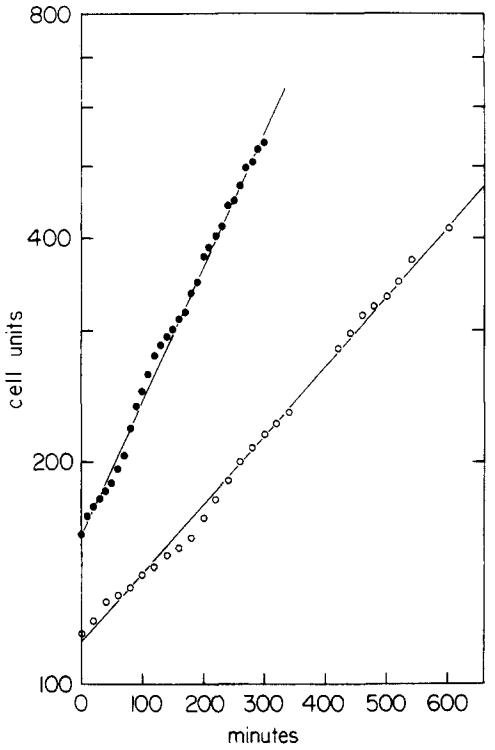

FIGURE 1 Growth of cells on solid medium. Cells of strain C276 growing on YNB-agar medium at 23°C without (●) or with 0.060 μg/ml cycloheximide (○) were photographed at successive intervals as described in Materials and Methods, and the increase in cell units (each parent cell and each bud is counted separately) was determined as a function of time.

medium at 23°C with a doubling time of 167 min in the absence of cycloheximide and 321 min in the presence of 0.060 μg/ml cycloheximide. The slight deviations from exponential growth may be a consequence of statistical fluctuations resulting from the limited sample sizes or may be due to a small perturbation in the cells resulting from the culture transfer.

To investigate the distribution of generation times of individual cells, the intervals between successive budding events of initially unbudded cells from the exponentially growing culture were scored. The first generation time of the parent cell (*P1* in Fig. 2) was equated to the interval of time from the appearance of its first bud until the appearance of its second bud, and the second generation was the interval from the parent cell's second bud until its third bud (*P2* in Fig. 2). The histograms of first and second generation times were unimodal (Fig. 3) with means of 132 ± 26 min and 138 ± 25 (here and elsewhere, standard deviations are given) min, respectively. Because the cells undergoing the first generation in this experiment included cells that are budding for their first time as well as cells (in decreasing proportion) that are budding for their second, third, etc., time and because the histograms for first and second generation are unimodal and approximately the same, we are justified in concluding that parent cells (cells that have a bud or have produced one or more buds) have approximately the same generation time for at least their first two to three cycles.

It is customary to compute generation times from one division to the next. Our method of utilizing the appearance of buds as the boundaries

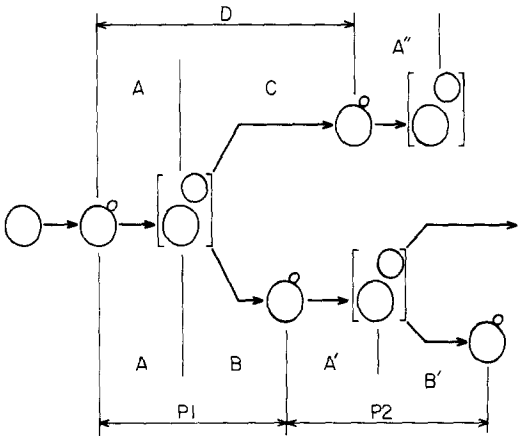

FIGURE 2 Definition of parent and daughter generation times from time-lapse photomicrographs. An initially unbudded cell (whose origin as a daughter or a parent from a previous cycle is unknown) is observed to bud. After some interval, defined as the first parent generation (*P1*), the parent cell buds for a second time. The daughter buds next, marking the end of the daughter generation (*D*). The second parent generation (*P2*) is defined as the interval from the parent cell's second budding until its third. Division of the parent from the bud occurs after interval *A* in the first parent generation and after interval *A'* in the second; division of the daughter from its first bud occurs after interval *A''*. The parent and daughter cells are separated in the diagram after division for clarity, but they actually remain together on the agar surface; consequently, the divisions which are shown in parentheses cannot be seen in the photographs.

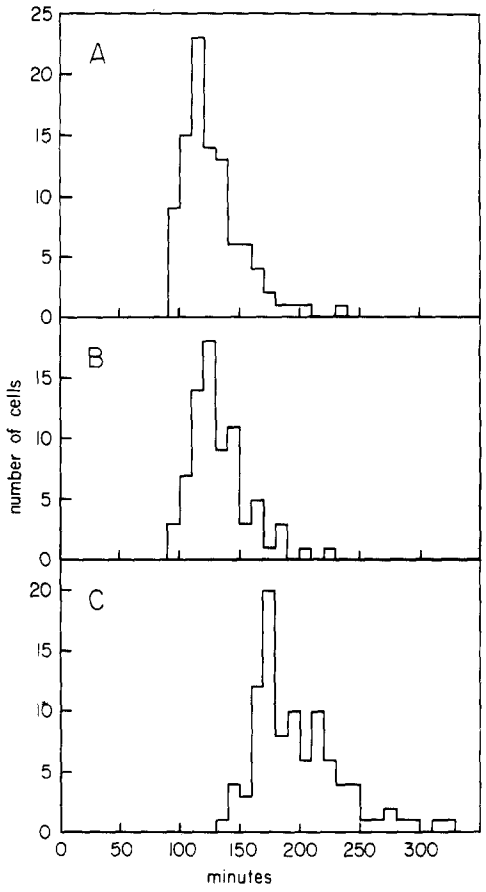

FIGURE 3 Histogram of parent and daughter generation times. Cells of strain C276 growing on YNB agar plates at 23°C without cycloheximide were photographed at 10-min intervals, and the intervals between successive budding events were scored from the photographs. Panel A is for the first parent generation, panel B for the second parent generation, and panel C for the daughter generation.

separating generations is necessitated by the fact that the time of division of the cells cannot be determined from photographs. Inspection of the diagram in Fig. 2 reveals, however, that the interval from the appearance of the parent's first bud until its second is identical to the interval from the division of the parent from its first bud until the division of the parent from its second bud, providing a parent cell has the same generation time (and the same allocation of this time to pre- and post-budding states) in generation n + 1 as it had in generation n (i.e. in Fig. 2, interval A = interval A', and hence interval $A + B$ = interval $B + A'$).

The generation time of the daughter is defined as the interval from its first appearance as a bud on the parent cell until it produces a bud of its own (interval D, Fig. 2). Inspection of Fig. 2 reveals that this interval is identical (with the same proviso as above) to the interval from the division of the daughter from the parent cell until its first division as a parent from its first bud (i.e. in Fig. 2 interval A = interval A'', and therefore interval $A + C$ = interval C + A"). The generation time for the daughter is also unimodal with a mean of 203 ± 38 min (Fig. 3). The generation time of the daughter is significantly longer than that for the parent cell, and the overall doubling time of the population must be a composite of these two.

A simple model of the cell cycle that accounts for these observations is presented in Fig. 4. We assume that all parent cells have the same generation time regardless of the number of daughters that they have produced previously. Further, we assume that daughter cells have a longer generation time during their first cell cycle that is accounted for entirely by the period before the time that they first bud. This formulation of the *S. cerevisiae* cell cycle was suggested previously (17), and we will present quantitative data in its support.

The standard age distribution equation (26) does not apply to a system undergoing unequal division, and a different formulation must be employed (see Appendix). The age distribution equation for the model presented in Fig. 4 can be used to derive a relationship between the generation time of the parent cell, the generation time of the daughter, and the population doubling time (see Appendix, Eq. 8). Solution of this equation for the population doubling time by numerical approximation gives a value of 165 min, and the agreement with the observed value of 167 min (Table I) is strong support for the model of Fig. 4. As a second test of this formulation, we have

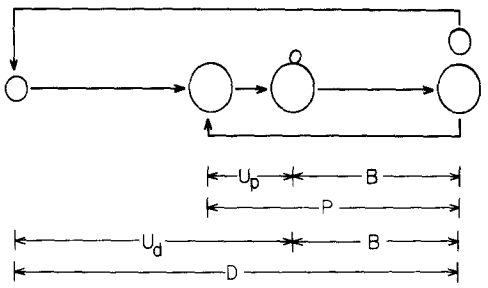

FIGURE 4 Model of the *S. cerevisiae* cell cycle. Abbreviations are as follows: U_p, parent cell unbudded period; B, parent cell budded period; P, parent cell generation time; U_d daughter cell unbudded period; B, daughter cell budded period; D, daughter cell generation time.

TABLE I

Parent, Daughter, and Population Generation Times for C276 Cells Growing Exponentially in Limiting Concentrations of Cycloheximide

	Generation time				
				Population	
Cycloheximide	1st Parent	2nd Parent	Daughter	Observed	Calculated
μg/ml					
0	127 ± 24		182 ± 23	155	153
0	132 ± 26	138 ± 25	203 ± 38	167	165
0.020	165 ± 26	145 ± 21	300 ± 41	220	226
0.030	171 ± 27	155 ± 29	328 ± 45	245	241
0.060	215 ± 50	193 ± 40	418 ± 67	321	305

The 1st and 2nd parent generation times and the daughter generation times were determined by time-lapse photography as defined in Fig. 2 and described in the legend of Fig. 3. The observed population doubling time was determined as in Fig. 1 and the calculated population doubling time was computed by substituting the first parent generation time and the daughter generation time in Eq. 8 (Appendix).

calculated the expected proportion of unbudded cells in this exponential population that are buds, i.e. have not budded before, and have measured this quantity by staining cells for bud scars. Unbudded buds were distinguished from unbudded parent cells in that the former have no bud scars while the latter contain one or more. The calculated value was 82% (see Appendix, Eq. 9) and the observed value was 78.8 ± 2.0%; the agreement with expectation was considered satisfactory.

Population Dynamics under Limiting Protein Synthesis

We have determined the generation times of parent and daughter cells when growth was limited by low concentrations of cycloheximide (Fig. 1). Cells were pregrown for 18–24 h in growth-limiting concentrations of cycloheximide and then followed by time-lapse photomicroscopy. The population doubling time, the parent generation time, and the daughter generation time were determined.

The parent and daughter generation times increase with increasing concentrations of cycloheximide, as does the population doubling time (Table I). The assumption in the model of Fig. 4 that all parent cells have the same generation time is supported by the observation that the first and second parent generation times are in reasonably good agreement although the second generation may be slightly faster than the first at the slower growth rates. The calculated population doubling time agrees reasonably well with the observed doubling time, and this result suggests that even under limiting growth conditions the model of Fig. 4 is valid. A further test of the validity of the model of Fig. 4 under conditions of limiting growth is provided by a comparison of the observed frequency of parent cells (those with one or more bud scars) among the unbudded cells with the frequency expected from the age distribution equation (Appendix, Eq. 9). The observations are in satisfactory agreement with expectation (Table II).

It is possible to separate the generation times into two intervals, the budded and the unbudded intervals. We have measured the frequency of budded cells in populations growing asynchronously on agar plates containing various concentrations of cycloheximide by washing the cells off the plate and counting the budded and unbudded cells. In the same experiment the population doubling times were determined. The fraction of budded cells can be converted to the interval of time

TABLE II

Percent of Unbudded Cells with No Bud Scar Compared to That Expected

Population doubling time	Observed*	Calculated‡
165	78.8 ± 2.0	0.82[a]
190	78.5 ± 2.1	
220	86.4 ± 1.7	0.79[b]
250	83.0 ± 1.9	0.79[c]
300	77.7 ± 2.4	0.74[d]
460	84.3 ± 1.8	

* Observed values were obtained using cells washed off of plates that contained various concentrations of cycloheximide after determining their population doubling times by time-lapse photography.

‡ Calculated values were obtained by use of Eq. 9 (Appendix) for the experiments of Fig. 5 in which both parent and daughter generation times had been determined. The population doubling times for the experiments of Fig. 5 were not identical to those obtained in this experiment but were close enough to warrant comparison and were as follows: *a*, 167 min; *b*, 220 min; *c*, 245 min; and *d*, 321 min.

that the average cell spends in the budded period under the model of Fig. 4 by using Eq. 3 (Appendix). The budded interval is relatively constant despite the changing growth rates (Fig. 5), and an empirical relationship derived by linear regression of the data in Fig. 5 between the length of the budded interval in minutes and the population doubling time is:

$$B = 0.17T + 87.4.$$

This equation was used to calculate the budded intervals in those experiments in which detailed data were obtained on parent and daughter generation times. By subtraction, we obtained the unbudded interval for parent and daughter (Fig. 5). Empirical relationships derived by linear regression of the data in Fig. 5 between the unbudded intervals and the population doubling time (in minutes) are as follows:

$$U_p = 0.36T - 42.0$$

$$U_d = 1.24T - 115.$$

In contrast to the budded period, the unbudded intervals are greatly prolonged as the growth rate is depressed. From the slopes of the curves in Fig. 5, it is evident that most of the increased generation time at slower growth rates is due to the increase in the unbudded intervals.

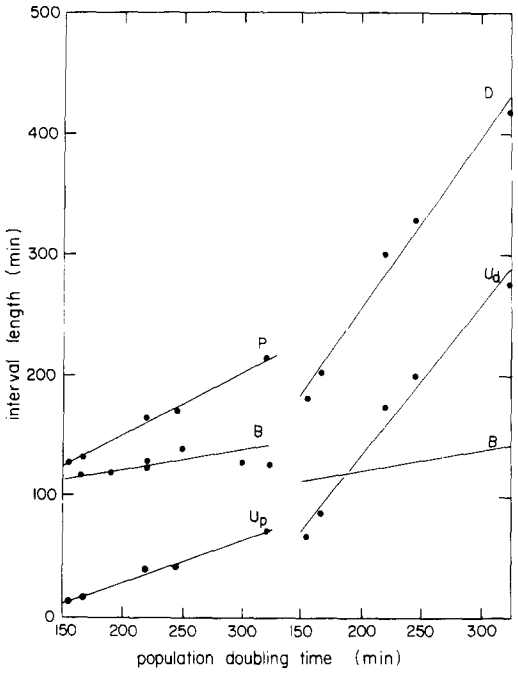

FIGURE 5 Cell cycle intervals as functions of population doubling time. Data for the generation times are taken from Table I. The data for the budded interval were derived in separate experiments as described in the text, and the data were fit by linear regression; the parent and daughter budded intervals (B) are assumed to be identical under the model of Fig. 4. The left panel presents data for parents and the right panel presents data for daughters. Abbreviations are the same as in Fig. 4. One point giving a budded interval of 176 min at a population doubling time of 460 min was used in the linear regression but is not shown on the graph. The parent and daughter unbudded intervals, U_p and U_d, respectively, were derived by subtracting the value for the budded interval (taken from the linear regression line) from the observed generation time. The lines for U_p and U_d were calculated by linear regression.

Relation of Growth to Division

The parent cell changes little in volume over the course of the budded interval. The parent portion of budded cells was measured for cells growing exponentially in YNB liquid medium without cycloheximide, and the volumes were computed for 29 parents with small buds (0.0–0.05 the volume of the parent) and for 20 parents with large buds (between 0.55 and 0.75 the volume of the parent; these are the largest buds present). All of the cells had a single bud scar (in the neck between parent and bud) and hence were in their first parental cycle. The average volume was 78.6 ± 12.4 μm^3 for cells with small buds and 84.6 ± 9.4 μm^3 for cells with large buds. Thus, the parent cell changes relatively little in volume over the course of the budded interval.

The temporal relationships between the unbudded interval, the budded interval, and the generation times of daughter and parent lead to certain expectations for the size of cells. Because the length of the budded interval is relatively independent of growth rate, and because the parent cell changes little in volume over the course of the budded interval, one would expect the size of the bud at the time of division to become progressively smaller at slower growth rates. This occurs because the cell devotes almost the same amount of time to the production of a bud whether it is growing rapidly or slowly. Even at the fastest growth rates encountered in these experiments, the bud does not reach the size of the parent cell at division. This conclusion was arrived at from two sets of observations necessitated by the fact that the photographic resolution of cells growing on agar is not sufficient to permit accurate measurement of cell size, and by the fact that in cells removed from liquid for high resolution phase-contrast microscopy, the identity of parent and bud cannot be determined. First, a naive observer was asked to tell which of the two units in a parent-daughter complex selected from time-lapse photographs to be at the time of division (10 min before the next budding of the parent cell) was the larger. In 68 out of 70 cases the observer picked the parent as the larger, in two cases the observer said that they were about the same, and in no case did the observer say that the bud was bigger. From this result, we felt justified in assuming that the larger component of a parent-daughter complex was the parent cell, and measurements were then made on cells growing in YNB liquid meduim where high resolution phase-contrast photographs could be obtained, but where the identity of the parent could not be determined. In a control culture growing with a population doubling time of 200 min, the volumes of the parent portion and bud portion of 394 budded cells were determined and the ratio of bud volume to parent cell volume was computed and plotted as a histogram. The parent portion of the budded cells had a mean of 96.2 ± 18 μm^3. As an estimate of the size of the bud at division, we take the value of the bud to parent volume ratio at the 95th percentile of the histogram, i.e. the value of the bud to parent volume ratio that was as great as that observed for

95% of the population, which was 0.77. The same measurement was made for 265 cells growing exponentially in 0.060 μg/ml cycloheximide at a doubling time of 348 min. The parent portion of the budded cells had a mean volume of 109 ± 33 μm^3, and the bud to parent volume ratio at the 95th percentile was 0.43 for this culture. Hence the bud is significantly smaller than the parent cell at the time of division in the control culture. Furthermore, when the growth rate is slowed by limiting the rate of protein synthesis, the bud becomes smaller at the time of division.

The data from the time-lapse experiments (Fig. 5) indicate that the parent cell has a detectable unbudded period at fast growth rates, and that this interval becomes progressively longer at slow growth rates. This fact suggests that the parent cell might become progressively larger each time it produces a bud. We have measured the volume of the parent portion of budded cells that were growing in medium with a generation time of 200 min and correlated these measurements with the number of bud scars on the parent cell (Table III). The data indicate that parent cells increase in volume by an average value of about 23% each generation. Since this is much larger than the amount of increase exhibited by a parent cell during the budded period (7%), most of this increase must be occurring during the unbudded interval.

Breakdown of the Unbudded Interval into Pre- and Post-α-Factor Execution

The point of mating factor arrest is the first known step in the cell cycle and is the point at which nutritionally limited cells arrest (2, 30, 35, Pringle and Maddox, personal communication) and the point at which growth and division are integrated (18). It was important therefore to determine how the increased length of the unbudded interval that occurs during growth limitation with cycloheximide is apportioned between the time before and the time after the point of mating factor sensitivity.

Haploid cells of strain 2180a were grown in low concentrations of cycloheximide for 24–48 h to achieve a steady state. They were then placed on solid medium containing α-factor and photographed at successive time intervals. Cells that were originally unbudded either remained unbudded and produced morphologically altered cells termed schmoos, or budded to produce two cells both of which then produced schmoos. The former class was considered to be before and the latter was considered to be subsequent to the point of α-factor arrest at the time of the shift. The interval of the unbudded period that precedes and succeeds the point of α-factor arrest is recorded in Table IV for a variety of growth rates. The former varies more than sixfold over the growth rates examined while the latter varies 1.5-fold. Consequently, the dramatic increase in the unbudded period that occurs at slower growth rates (Fig. 5) occurs almost exclusively in the unbudded interval before the point of mating factor arrest.

TABLE III

Volumes of the Parent Portions of Budded Cells as a Function of the Number of Bud Scars That They Possess

No. scars	No. cells	Mean volume
		μm^3
1	217	79.4 ± 15.2
2	101	108.4 ± 20
3	33	119.9 ± 20
4	21	147.6 ± 19

Other Protein Synthesis Inhibitors

We wished to determine whether the preferential lengthening of the unbudded phase of the *S. cerevisiae* cell cycle by cycloheximide was a general response to a limitation of protein synthesis or a specific response to this inhibitor. A number of inhibitors and temperature-sensitive mutations

TABLE IV

Length of the Unbudded Period That is Located before and subsequent to the Point of α-Factor Arrest for Different Growth Rates

Population doubling time	Length of unbudded interval		
	Total*	Before‡ α-factor	After§ α-factor
min			
198	122	98	24
324	248	218	30
492	409	387	22
714	656	636	20

* Calculated from the fraction of the cells that were unbudded, using Eq. 3 (Appendix).

‡ The interval of the unbudded period that proceeded the point of α-factor arrest. Calculated from the proportion of unbudded cells that failed to divide in the presence of α-factor.

§ The interval of the unbudded period that suceeded the point of α-factor arrest. Calculated as the difference between the second and third columns.

that are known to block protein synthesis in *S. cerevisiae* were tested to see whether depressed, exponential growth rates could be attained at moderate levels of inhibitor or intermediate temperatures. We were able to attain steady-state conditions for the inhibitors mimosine (29) and trichodermin (32), for the aminoacyl tRNA synthetase mutations, ils 1 (13) and mes 1 (22), and for the mutation prt 1 which blocks the initiation of polypeptide chains (14). The temperature-sensitive protein synthesis mutants were grown at a variety of temperatures, and the inhibitor-sensitive strains were grown in different concentrations of inhibitor; the growth rate as well as the fraction of budded cells was determined. The length of the budded period was then calculated, using the age distribution equation (Appendix, Eq. 3). A plot of the increase in the length of the budded period as a function of the increase in generation time is recorded in Fig. 6. Included in these data are experiments in which cycloheximide was used as growth inhibitor for three different strains. We have not attempted to designate each strain and growth limitation because all strains behaved similarly. The result in all cases was that the length of the budded period changed relatively little with increasing growth rates. The linear regression line through these points has a slope of 0.17 min/min for the rate of change of the budded period as a function of population doubling time, a value that is identical to that obtained for strain C276 growing on solid medium containing various concentrations of cycloheximide (Fig. 5). It is possible that curves other than a straight line would provide a better statistical fit to the data, but we have not investigated this possibility. Therefore, the major consequence of a limitation of growth at the level of protein biosynthesis is a lengthening of the unbudded interval of the cycle.

DISCUSSION

We have examined the growth and division of *S. cerevisiae* cells under steady-state conditions when the rate of protein synthesis was growth rate limiting. We observe that the cells divide unequally: the bud is smaller than the parent cell at division, and the length of the next cycle is longer for the bud than for the parent. These inequalities become more pronounced as the rate of protein synthesis is depressed. Furthermore, the parent cell remains relatively constant in volume throughout the budded portion of the cycle, and the length of the budded interval varies only slightly as the growth rate is depressed. Although a systematic investigation of the cycle times of parent and bud at different growth rates has not been reported previously, it is worth noting that each of these five observations has been reported numerous times (see the discussion of reference 18 for an exhaustive review on the relationship between growth and division of *S. cerevisiae* cells), and there can be no doubt about the generality of these phenomena under a variety of conditions. Two particularly pertinent prior studies are those of Von Meyenburg (31) and Barford and Hall (1). Von Meyenburg found that the proportion of unbudded cells increased dramatically as the generation time increased in glucose-limited chemostats

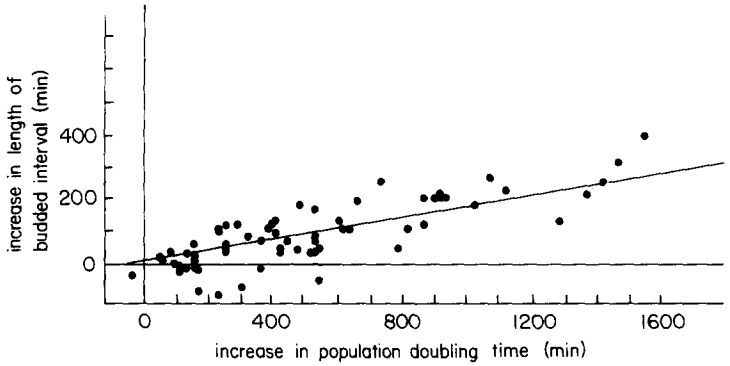

FIGURE 6 Length of the budded interval as a function of growth rate in the presence of various inhibitors or mutations that limit growth. The data were obtained with a variety of strains, inhibitors, and mutations as indicated in Materials and Methods. Since different strains exhibited different population doulbing times in the control (no inhibitor), we have plotted the increase in length of the budded interval (the length of the budded interval in the uninhibited culture was subtracted from each value) as a function of the increase in length of population doubling time. The line is calculated by linear regression of the points.

and concluded that all of the increase in generation time was occurring during the unbudded interval of the cell cycle. Barford and Hall noted a greater than 20-fold lengthening of the G1 interval for cells growing on ethanol compared to those growing on glucose; the increase in G1 accounted for most of the increase in generation time.

The results reported herein are, at least qualitatively, what would be expected from a model presented previously for the coordination of growth and division in *S. cerevisiae* (18). The model proposed that growth rather than progress through the DNA-division cycle is normally rate-limiting for cell proliferation and that a critical cell size must be attained before the completion of the start event in G1. Because the length of the budded phase does not change markedly with growth rate, it is apparent that a parent cell has about the same amount of time to produce a bud at slow growth rates as it does at fast growth rates. Furthermore, because the parent cell remains relatively constant in volume throughout the budded phase, essentially all of the growth that occurs during this time is apportioned to the bud at division. It follows that the size of the bud should be smaller at division for cells growing more slowly. This is what we observe; the bud was estimated to have a volume 0.77 that of the parent cell at division for cells growing with a doubling time of 200 min, and 0.43 for cells with a 348-min doubling time. If the cell must attain a critical size before it can begin a cell cycle, then we would expect the daughter (bud) to have a longer unbudded phase than the parent cell, and the length of the unbudded interval should increase as the growth rate decreases. This expectation is also fulfilled by the observations.

It would be even more satisfying if we could test the quantitative agreement between our data and expectation. To make a quantitative comparison, however, one must make some ad hoc assumptions about the way in which individual cells grow. If we assume that individual cells increase their masses exponentially with the same rate constant throughout the cell cycle, then the constant must of course be the same as that for the population as a whole. We can then ask whether the observed time intervals for unbudded and budded periods are consistent with the maintenance of a steady state in the culture with respect to cell growth and division. For example, if we assign a cell that is budding for the first time a mass m_o, then the daughter of this parent must reach mass m_o at the time it buds for its first time. This necessity arises from the fact that the cells are growing under steady-state conditions and is not dependent upon any particular models for growth or division.

For the discussion that follows it is more convenient to consider the mass, m_p, of a daughter cell that is P time units from the next division (this point in the cycle will be called the reference time; see Fig. 4). This cell has grown for $D - P$ time units since the last division, and will bud in $P - B$ time units, where P is the parent cell generation time, D is the daughter cell generation time, and B is the length of the budded interval. The steady state assumption demands that the daughter of this cell also reach mass m_p after D time units have elapsed. If all of the mass increase that occurs after this cell buds is distributed to its daughter bud at division, then the new daugher will have a mass of $m_p e^{\alpha(D-B)} (e^{\alpha B} - 1)$ at the reference time in the next cell cycle. This expression has been evaluated in Table V (model 1) for the five different growth rates, and it is evident that the steady state is not maintained under this set of assumptions, especially at the slower growth rates. That is, for all growth rates the mass of a new daughter at the reference time is considerably less than the mass of its parent one cycle earlier. Of course, any one of our assumptions about the way the individual cells grow or apportion mass between daughter and parent could be altered to accommodate the steady state. We will present only one possible change that has an interesting biological implication. If we assume that a parent cell contributes to its bud at division, the mass accumulated during the entire P interval (rather than just the mass accumulated during the B interval), then the mass of the daughter at the reference time would be $m_p e^{\alpha(D-P)} (e^{\alpha P} - 1)$. Evaluation of this expression for the different experiments shows reasonably satisfactory agreement with the steady-state as-

TABLE V

*Predicted Mass in Units of m_p of a Daughter Cell That is P Time Units from the Next Division**

Population doubling time	Model 1	Model 2
155	0.895	0.978
167	0.887	0.980
220	0.838	1.043
245	0.773	0.970
321	0.645	0.902

* Values for P, B, and D are taken from Fig. 5.

sumption (Table V, model 2). Hence one way of maintaining the steady state is for the parent cell to contribute the mass it accumulates during its unbudded period (in addition to that accumulated during its budded period) to its daughter at division. However, another difficulty arises with this assumption. If the parent cell contributes all of its mass increase to the daughter cell, then the parent cell would not be expected to increase in size in successive generations. But we observe, as have others (16, 21, 24), that the parent cell does increase in volume each generation (Table IV).

These difficulties in accounting quantitatively for the growth of individual cells may be more apparent than real as a consequence of our ignorance regarding how the cell measures its size. It is fairly clear that growth is necessary specifically for completion of the first step in the cell cycle, the step that is sensitive to mating factor and is controlled by the *cdc* 28 gene product. For reasons of convenience, we have used time and volume as measures of this growth requirement in these experiments and total mass or protein content in other studies (18). These gross parameters of cell size are not well coordinated during the cell cycle (11, 23, 33), and it is likely that the cell is monitoring some event other than these (like the amount of one specific protein) that may be only loosely correlated with volume, mass, and total protein content. In fact, a histogram for cellular volume of the parent portion of cells with small buds that are in their first parental generation is quite broad with a mean of 76.0 ± 14.3 μm^3 (data not shown). Clearly, volume itself is not the parameter that the cell monitors. In short, a qualitative consideration of the data is all that appears to be warranted at the present time.

Arrest of cell division at the start event is also observed when prototrophic cells are starved for any one of a variety of nutrients (2, 30, 35, Pringle and Maddox, personal communication). An attempt to locate a signal in the form of a metabolic intermediate of the sulfate assimilation pathway led to the conclusion that if a single signal existed it must be at or subsequent to methionyl-tRNA (30). The fact that accumulation of cells before the start event(s) occurs under a variety of conditions that limit polypeptide initiation and elongation suggests that the controlled response to nutritional starvation and the mechanism for maintaining size homeostasis may be one and the same. At the current state of our understanding, both of these phenomena can be explained by assuming that some particular protein, e.g., the initiator substance of Donachie (7), is made at a constant differential rate of total protein synthesis and that a sufficient amount of this protein must accumulate to permit completion of the start event. Other models are also tenable.

APPENDIX

Consider an asynchronous, exponentially multiplying cell population in which cells progress through the cycle as diagrammed in Fig. 7. The number of cells, $N(t)$, present at any time, t, is given by

$$N(t) = N(0)e^{\alpha t}, \tag{1}$$

where $\alpha = \ln 2/T$, T being the population doubling time.

The position of a particular cell in the cell cycle is defined as the time, τ, it will take that cell to reach division. Thus, τ is a metric of the age of a given cell, has a value of 0 at division, increases from right to left along the abscissa of Fig. 7, and has a maximum value of D, the daughter cell generation time. We shall assume that there is no dispersion in the daughter or parent cell generation times. Although the data (Fig. 3) demonstrate a dispersion of measured generation times as well as a skewness to longer generation times, we ignore these complications for two reasons. First, the simpler model is mathematically more tractable, and seond, we cannot assess how much of the dispersion is due to the behavior of the cells and how much is introduced by the measurement procedure (the photographs were not always of perfect clarity, and they were taken at 10- to 20-min intervals). Furthermore, the simple model appears to be adequate since the predictions made by it are in good agreement with the data (Tables I and II).

Let $g(a,b)$ be the number of cells contained in an interval of the cycle between $\tau = a$ and $\tau = b$ at time $t = 0$. All of the new cells produced in the population during an interval of time, t, where $t < P$ (the parent cell generation time), arise by division of cells that lie at time $t = 0$ in the interval of the cycle between $\tau = 0$ and $\tau = t$.

$$N(t) - N(0) = g(t, 0), \tag{2}$$

where $0 \leq t < P$.

From Eq. 1 and letting $\tau = t$,

$$g(\tau, 0) = N(0)[e^{\alpha\tau} - 1], \tag{3}$$

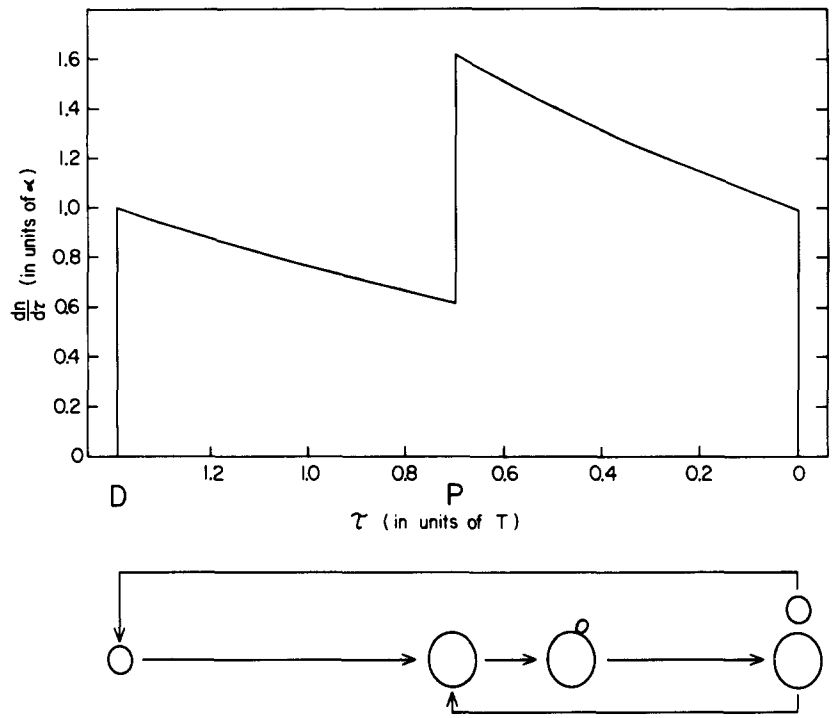

FIGURE 7 The age distribution of cells undergoing unequal division. Axes and symbols are defined in the text.

where $0 \leq \tau < P$.

The number of cells per unit of time at time $t = 0$ passing through a point, τ, in the cycle is represented on the ordinate of Fig. 7 and for the interval $0 \leq \tau < P$ is:

$$\frac{dg(\tau, 0)}{d\tau} = N(0)\alpha e^{\alpha\tau}, \tag{4}$$

where $0 \leq \tau < P$.

To derive the equation for the ordinate of Fig. 7 for $\tau > P$, we must consider the origin of new cells in the population for $t > P$. The increase in cell number during the interval between time P and t where $t > P$ will result from the division of cells located at time $t = 0$ in the interval $\tau = P$ to $\tau = t$ plus the division (for the second time) of cells located at time $t = 0$ in the interval $\tau = 0$ to $\tau = t - P$,

$$N(t) - N(P) = g(t, P) + g(t - P, 0). \tag{5}$$

Substituting from Eqs. 1 and 2 and letting $\tau = t$,

$$g(\tau, P) = N(0)e^{\alpha\tau}[1 - e^{-\alpha P}] - N(0)[e^{\alpha P} - 1], \tag{6}$$

for $P < \tau < 2P$.

The number of cells per unit of time at time $t = 0$ passing through a point, τ, in the cycle for $P < \tau < 2P$ is:

$$\frac{dg(\tau, P)}{dt} = \alpha N(0)e^{\alpha\tau}[1 - e^{-\alpha P}], \tag{7}$$

for $P < \tau$.

A similar argument for intervals $2P < \tau < 3P \ldots nP < \tau < (n + 1)P$ demonstrates that Eq. 7 is valid for all $\tau > P$.

Thus, Eqs. 4 and 7 describe $\frac{dg}{d\tau}$ (the ordinate of Fig. 7) as a function τ (the abscissa) for $0 \leq \tau < P$ and $P < \tau$, respectively.

If we set $N(0) = 1$, the value of $dg/d\tau$ at any point, τ, represents the frequency of cells per unit time passing through τ. The value of $dg/d\tau$ can be obtained from Eqs. 4 or 7 for appropriate values of τ. $dg/d\tau$ at $\tau = 0$ is the frequency of cells undergoing division per unit time and has a value from Eq. 4 of α; $dg/d\tau$ at $\tau = P$ from Eq. 4 represents the frequency of cells immediately to the right of the point where parent cells reenter the cycle after division and has a value of $\alpha e^{\alpha P}$;

$dg/d\tau$ at $\tau = P$ in Eq. 7 represents the frequency of cells immediately to the left of the point where parent cells reenter the cycle after division and has a value of $\alpha e^{\alpha P}\,[1 - e^{-\alpha P}]$; $dg/d\tau$ at $\tau = D$ is the frequency of daughter cells immediately after division and has a value from Eq. 7 of $\alpha e^{\alpha D}\,[1 - e^{-\alpha P}]$. Since the frequency of cells at division must equal the frequency of daughter cells immediately after division, $\alpha e^{\alpha D}\,[1 - e^{-\alpha P}] = \alpha$,

$$e^{\alpha D}[1 - e^{-\alpha P}] = 1. \tag{8}$$

Since $\alpha = \ln 2/T$, Eq. 8 is the relationship between the parent generation time, the daughter generation time, and the population doubling time.

With *S. cerevisiae* it is possible to distinguish daughter cells (who lack bud scars) from parent cells (with bud scars) and it is useful therefore to derive their expected frequencies according to the theory of Fig. 7.

For example, the proportion of unbudded cells that are daughters, $u(d)$, and hence have no bud scar, is found by integrating Eq. 7 between D (the daughter cell generation time) and B (the length of the budded period) and dividing this result by the total number of unbudded cells. The later quantity is found by integrating Eqs. 4 and 7 between appropriate limits:

$$u(d) = \frac{\int_B^D \alpha e^{\alpha\tau}[1 - e^{-\alpha P}]d\tau}{\int_P^D \alpha e^{\alpha\tau}[1 - e^{-\alpha P}]d\tau + \int_B^P \alpha e^{\alpha\tau}d\tau} = \frac{[1 - e^{-\alpha P}][e^{\alpha D} - e^{\alpha B}]}{[1 - e^{-\alpha P}][e^{\alpha D} - e^{\alpha P}] + [e^{\alpha P} - e^{\alpha B}]}. \tag{9}$$

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