Asymmetrical Division of *Saccharomyces cerevisiae* in Glucose-limited Chemostat Culture

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*Saccharomyces cerevisiae* S288C/1 was grown in a glucose-limited chemostat at population doubling times ($\tau$) of 80 to 736 min. Estimates of the daughter cycle time (D), the parent cycle time (P) and the budded period (B) were obtained from bud scar analyses and equations derived from the Hartwell & Unger model of asymmetric cell division. D, P and B all showed biphasic linear relationships to $\tau$, quantitatively different from estimates for the same strain in batch culture. Median cell volume and dry weight per cell increased at the faster growth rates, but the average cell density reached a minimum at $\tau = 150$ min. The contiguous array of bud scars on parent cells became increasingly irregular as the doubling time increased from 140 min. At the fastest growth rates a small percentage of filamentous forms were observed due to failure of cells to undergo cell separation.

INTRODUCTION

Using data from time-lapse cinematographic experiments, Hartwell & Unger (1977) suggested that *Saccharomyces cerevisiae* underwent an asymmetric cell division. They proposed a specific model in which (i) cells needed to attain a critical cell size to initiate the cell cycle at start, and (ii) as the growth rate decreased daughter cells were born at an increasingly smaller size than the parent cells. The daughter cycle time (D) would thus need to be longer than the parent cycle time (P) for daughter cells to achieve this critical cell size. D and P are related to the population doubling time ($\tau$) by

$$e^{-aD} + e^{-aP} = 1$$

where $\alpha = \ln 2/\tau$. \hfill (1)

This model has received strong experimental support from data on bud scar analyses (Carter & Jagadish, 1978; Lord & Wheals, 1980). A bud scar is a chitin ring deposited at the bud isthmus during bud formation and left on the parent at cell separation. It is possible to visualize these scars using fluorescent dyes such as Primulin and Calcofluor (Streiblova & Beran, 1963; Hayashibe, 1977) and hence determine the genealogical age of a cell. A new-born daughter will have no bud scar (genealogical age 0), a parent cell that has budded once will have one bud scar (genealogical age 1) and so on. By measuring the fraction of budded and unbudded cells of different genealogical ages it is possible to compute values of D, P and B, the budded period. It was shown (Lord & Wheals, 1980) that all three parameters varied linearly with growth rate in batch culture and that asymmetrical divisions occurred at all growth rates. Equations were developed to describe the genealogical age distribution of asymmetrically dividing yeast cells and excellent agreement was found between theory and experiment (Lord & Wheals, 1980). Previous observations of an increasing fraction of daughter cells at slow growth rates and different fractions of cells of different genealogical ages varying with growth rate (Beran, 1968; Carter & Jagadish, 1978; Vrana, 1976; Beran et al., 1966) could now be explained.

Analysis of the cell cycle via manipulation of the growth rate is a useful method for under-
standing temporal controls. Both batch and chemostat culture methods have been used and the implicit assumption has been that the results obtained are equivalent. As yet, there has been no comprehensive comparison of the results obtained for the same strain at the same temperature by these two methods of doubling time manipulation. This paper reports a chemostat culture analysis of a strain that has been extensively studied in batch culture (Tyson et al., 1979; Lord & Wheals, 1980) in order to see how far the predictions of the Hartwell & Unger model apply to chemostat-grown cells, and whether the quantitative relationships obtained are similar to those of cells grown in batch culture.

METHODS

Organism. A wild-type haploid strain of *Saccharomyces cerevisiae* obtained from Dr C. F. Roberts, Genetics Department, University of Leicester, was used throughout. A derivative of S288C (Lord & Wheals, 1980), it is designated S288C/1.

Medium. The medium used contained 10 g yeast extract, 20 g bacteriological peptone and 20 g glucose per litre. It was filtered twice through Whatman GF/D and GF/F filters to remove any particles and then sterilized by autoclaving. Other chemicals were from BDH.

Growth conditions. Continuous cultivation was carried out in a CC1500 fermenter (LH Engineering, Stoke Poges, Bucks.) using a working volume of 2 l. The temperature was maintained at 30 °C, the air flow at 21 min⁻¹, the stirrer speed at 600 rev. min⁻¹, and the pH at 5.5 regulated by the addition of 1 M-HCl or 1 M-KOH. Foam formation was reduced by the addition of a 5 % (v/v) solution of silicone DC antifoam emulsion M10 (Hopkin & Williams, Chadwell Heath, Essex). After a change in dilution rate (equal to ρ), the culture equilibrated after 20 l of the medium had passed through the vessel when new measurements were taken.

Bud scar analyses. Cells were fixed in formaldehyde, stained with Calcofluor, examined under fluorescence microscopy and the presence or absence of a bud and the number of bud scars was noted for at least 1000 cells as described previously (Lord & Wheals, 1980).

Cell number and cell volume measurements. These were made on an electronic particle counter as described previously (Tyson et al., 1979).

Dry weight determinations. A 5 ml culture sample was filtered through a pre-dried, pre-weighed, 0.45 μm pore-size cellulose acetate Nulflow filter (Oxoid), washed several times in distilled water and dried to constant weight in a vacuum drying oven at 120 °C. Five samples were taken at each growth rate.

Estimates of D, P and B. These were calculated by the maximum likelihood method from the bud scar analysis data and the dilution rate (equal to In 2/τ) as described previously (Lord & Wheals, 1980).

Genealogical age distribution. The predicted frequency of cells of different genealogical ages can be calculated by substituting estimates of P into

\[ F_D = e^{-αP} \]  
(2)

for the fraction of daughter cells and

\[ F_{F(n)} = (e^{-αP})^{n-1} (1 - e^{-αP})\]  
(3)

for the fraction of parent cells of genealogical age \( n \), where \( n \) is equal to the number of bud scars (Lord & Wheals, 1980).

RESULTS

Asymmetrical age distribution

Estimates of the daughter cycle time (D), the parent cycle time (P) and the duration of the budded phase (B) were calculated using the dilution rate of the culture, the number of unbudded daughter cells, the number of unbudded parent cells, the number of budded cells, the total number of scars and the equations derived from the Hartwell & Unger model (Lord & Wheals, 1980). The values of D, P and B obtained were plotted against the population doubling time (τ) (Fig. 1). (The results are presented on a double logarithmic plot in order to accommodate the large number of points at the lower end of the scale.) For all three parameters there were two distinct phases, both of which were linearly related to τ (Table 1). It was suggested (Lord & Wheals, 1980) that the age distribution for asymmetrically dividing cells became the same as that for symmetrically dividing cells at the maximum balanced growth rate (i.e. at \( \mu_{max} = D = P = τ \)). If the lines of D and P at the slower growth
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Fig. 1. Daughter cycle time ($D$, ○), parent cycle time ($P$, ●) and budded period ($B$, □) as a function of the population doubling time ($τ$). The values were calculated by the method of maximum likelihood from bud scar analyses and the dilution rate. (Note that the scale is logarithmic on both axes.)

Table 1. Daughter cycle time ($D$), parent cycle time ($P$) and budded period ($B$) as a function of the population doubling time ($τ$)

<table>
<thead>
<tr>
<th></th>
<th>$D$</th>
<th>$P$</th>
<th>$B$</th>
<th>$P−B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>$1.04τ+9$</td>
<td>$0.96τ−8$</td>
<td>$0.71τ+6$</td>
<td>$0.25τ−14$</td>
</tr>
<tr>
<td>Slow</td>
<td>$1.71τ−128$</td>
<td>$0.50τ+82$</td>
<td>$0.11τ+108$</td>
<td>$0.39τ−26$</td>
</tr>
</tbody>
</table>

The values were obtained by linear regression, the correlation coefficients being better than 0.78 in all cases. The fast growth rates included values faster than $τ = 200$ min; the slow growth rates included values slower than $τ = 190$ min. All values are expressed in min.

rates are extrapolated, $D$ would equal $P$ and $τ$ at approximately 170 min. This did not occur because at doubling times less than 200 min a second phase occurred in which the fraction of daughter cells remained constant at approximately 55%. This is clearly shown in Fig. 2 where the ratio $D:P$ is plotted against the specific growth rate ($μ$). As $μ$ decreased from 0.2 to 0.05, the ratio of daughters to parents increased until at the slowest growth rates there are more than twice as many daughters as parents in the population. Since bud emergence is dependent on start of the cell cycle (Hartwell et al., 1974) and it has been suggested that start occurs at the birth of a parent (Lord & Wheals, 1980), a plot of $P−B$ against $τ$ would reveal whether the interval between these two events was constant or varied with growth rate. Figure 1 indicates that the $P−B$ interval increased linearly with doubling time (Table 1) except possibly at the fastest growth rates where it seemed to remain constant.

At doubling times less than 80 min, small, stable clumps of yeast cells were found constituting only 1 to 2% of the population. The individual cells could not be separated from each other by mild sonication which was routinely performed on all chemostat samples.

Genealogical age distribution

The method and theory of determining the genealogical age distribution have been described by Lord & Wheals (1980). The predicted values were calculated using the two estimates of $P$ in Table 1. Figure 3 shows the predicted variation in the percentages of cells
Fig. 2. Ratio of daughter (D) to parent (P) cycle time as a function of the specific growth rate ($\mu$).

Fig. 3. Relative frequency of cells of different genealogical ages as a function of the population doubling time ($\tau$). The curves are based on the numerical relationships of P to $\tau$ in Table 1 and equations 1 and 2.

Fig. 4. Relative frequency of parent cells of age 1 (●) and age 2 (○) as a function of the population doubling time ($\tau$). The data points were taken from measurements of over 1000 cells for each doubling time and the curves have been redrawn from Fig. 3.

of different genealogical age with $\tau$ based on these relationships. At doubling times greater than 200 min the genealogical age distribution varied due to the pronounced asymmetrical cell division. At doubling times less than 200 min there was little predicted change in any of the different age classes, as expected from Fig. 1. Experimental values of the percentages of cells of age 1 and age 2 are shown in Fig. 4. The data give a good fit to the predicted values for the asymmetrical division model and are quite different from the predictions of a
symmetrical division model where the percentages of $P_1$ and $P_2$ should remain constant at 25 and 12.5 %, respectively. An analysis of the remaining data using a 'box and whiskers' plot (Lord & Wheals, 1980) revealed a good fit to the predicted values at all growth rates except for a slight shortage of cells with ages greater than 4, and a corresponding excess of daughter cells.

**Cell volume, dry weight and density**

Median cell volume increased at doubling times faster than 300 min to give a twofold increase at the fastest growth rates (Fig. 5a). The dry weight and number of cells per unit volume were measured at all growth rates and the dry weight per cell was calculated (Fig. 5b). The shape of the curve is similar to that of the median cell volume measurements suggesting that the increase in volume was not an unspecified osmotic effect but a real effect of macromolecular synthesis.

The dry weight per cell was divided by the median cell volume to give median cell density (Fig. 5c), revealing a biphasic relationship of density to $\tau$. Cell density decreased at doubling times from 700 to 150 min and then sharply increased at the fastest growth rates.

**Position of the bud scars**

The position of the bud scars on cells growing at different doubling times was noted. At the faster doubling times virtually all cells had their bud scars at the same pole in a precisely ordered sequence, but as the doubling time increased so did the fraction of cells with bud scars at both poles, or with gaps in the sequence of scars (Figs 6 and 7). It was possible to deduce that gaps in the bud scar sequence were not filled in during subsequent cycles since the fraction of cells with disordered sequences always increased with increasing cell age (results not shown).
DISCUSSION

In batch culture experiments it has been shown that the Hartwell & Unger model (1977) of asymmetric cell division provided a quantitative explanation of the experimental estimates of D, P and B, and the observed values of $F_D$ and $F_P$ (Lord & Wheals, 1980). It was possible to test independently a number of assumptions of the model and demonstrate that they were valid. The same tests have been applied to the present data (analyses not shown) and the same general conclusions emerge. In populations of cells growing in a chemostat under balanced steady-state exponential growth conditions we conclude that, at any one growth rate, (i) division was asymmetrical (Fig. 1), (ii) $D > P > B$ (Fig. 1), (iii) $P$ was constant for cells of different genealogical age, (iv) $B$ was the same for daughters and parents (of all ages), (v) the genealogical age distribution was as predicted by the model (Fig. 4) and (vi) there was an increasing probability of death at increasing genealogical age.

This analysis revealed a clear biphasic linear relationship of $D$, $P$ and $B$ to $\tau$, the transition point being at about 200 min in all cases. The monophasic linear relationship found in batch culture studies was only extended to doubling times as slow as 250 min and thus may only have been relevant to faster growth rates (Lord & Wheals, 1980). Average cell density ($\rho$) also changes at a doubling time of about 200 min, reaching a minimum at this doubling time.

The transition point for $D$, $P$ and $B$ corresponds almost exactly to that found by Beck & von Meyenburg (1968) in their analysis of the level of certain enzymes of $S. cerevisiae$ in glucose-limited chemostat culture at 30 °C. At doubling times less than 200 min they concluded that the cells were growing by ‘aerobic fermentation’, while at slower growth rates ‘oxidative metabolism’ was occurring.

Figure 8 compares the values of $D$, $P$ and $B$ found in chemostat culture with those found in batch culture over the same range of doubling times (70 to 250 min). Apart from the
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![Graph](image-url)

**Fig. 8.** Daughter cycle time (D), parent cycle time (P) and budded period (B) as a function of the population doubling time (τ) over the range 70 to 250 min. The values are for batch-grown cells (---), redrawn from Lord & Wheals (1980), and for chemostat-grown cells (---), redrawn from Fig. 1.

Biphasic nature of the chemostat values, the main differences from batch culture are longer P and B periods and a shorter D period.

The relationship of D, P and τ shown in equation 1 reveals that once two of the parameters are set, the third can be calculated. Biologically, under balanced growth conditions when mass doubling time equals population doubling time, with τ set, it is P that determines D. This is because the daughter cycle time is determined by the period needed for a daughter to grow from birth size to critical size, at which parents are born (Johnston *et al.*, 1977). The birth size of the daughter is determined in turn by the time during which mass is synthesized in the parent cycle and its partitioning into the bud during the B period. Since B is a part of P, an elongation of B will also elongate P. The extended attachment of a bud to a parent will result in a larger daughter at birth, which will thus have a correspondingly shorter cycle time. The elongation of the B period and the interdependence of D, P, B and τ seem to be the principal reasons for the altered relationships seen in chemostat culture, where the B period can be as much as 50 min longer.

The cause of the elongation is unknown but it could be related to relief from catabolite repression. During exponential growth in batch culture the carbon substrate is present in excess while in chemostat culture the substrate is certainly growth-limiting at doubling times greater than 100 min (Beck & von Meyenburg, 1968). Such an explanation predicts that at the fastest growth rates, when the media in chemostat and batch culture are identical, each parameter should be identical in the two conditions. At τ = 80 min each value in batch culture is within 6 min of the value in chemostat culture.

The D and P curves for batch culture converge to equality at τ = 65 min. Although unattainable, it was predicted (Lord & Wheals, 1980) that at such a growth rate symmetrical division would occur. No such expectation was predicted from extrapolation of the chemostat data, yet symmetrical division was seen amongst a small percentage of cells when they produced filaments at τ < 80 min. The symmetry was revealed by the identity of size and the synchrony of bud emergence of parents and daughters. (A fuller account of this change of morphology will be presented elsewhere.) This observation again points to a similarity of behaviour of chemostat- and batch-grown cells at the fastest growth rates.

Cell volume differences were also seen between chemostat and batch cultures. *Saccharomyces cerevisiae* cells grown in either glucose-limited or ammonium-limited chemostats are larger at faster growth rates (McMurrough & Rose, 1967; Mor & Fiechter, 1968), as has
been found here where there is a twofold difference in median cell volume over the growth rates studied. More interestingly, the largest cells are still smaller than the smallest cells of the same strain grown at fast growth rates in batch culture (Lord & Wheals, 1980), in contrast to the identical volumes found in similar work by Johnston et al. (1979). The median cell volumes differ because the initial size for traverse of start is dependent on growth rate (Lord & Wheals, 1980). A similar nutrient-modulated size control over division has been seen in 

\textit{Schizosaccharomyces pombe} (Fantes & Nurse, 1977).

The conclusion from a comparison of batch- and chemostat-grown cells is clear. The biology of cells growing at the same growth rate can be quite different and caution should be exercised in extrapolating from one set of conditions to another.

Starting from the birth scar, buds on haploid cells are formed in highly ordered sequences as rings, rows or spirals (Streiblova, 1970; Freifelder, 1960). Cytoplasmic microtubules extend from the spindle pole body (SPB), embedded in the nuclear membrane, to the base of, and sometimes into, the emerging bud. After division, the SPB is at the opposite pole to both the birth scar in the daughter cell and the last bud scar in the parent cell, so that if the SPB controls the location of the new bud it will have to reorientate precisely at each cycle (Lord & Wheals, 1980). One possible source of spatial information could be the microtubules extending in from the last bud scar. At doubling times less than 136 min over 98% of the population showed this precise control but as the growth rate fell so did the degree of precision. The irregularities could appear at any genealogical age with the result that the oldest cells in the population had the highest probability of showing an irregular pattern.

From the results of this study it is proposed that the quantitative predictions of the Hartwell & Unger model (1977) can be applied to glucose-limited chemostat culture as well as to batch culture (Lord & Wheals, 1980). Although differences do exist between cells grown in batch and chemostat culture which affect the subsequent biology of the cell, these differences are quantitative not qualitative. The biphasic nature of Fig. 1 reinforces our view that aspects of the yeast cell cycle should be analysed over as wide a range of growth rates as possible.

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REFERENCES


