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Dependency of Size of *Saccharomyces cerevisiae* Cells on Growth Rate

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The mean size and percentage of budded cells of a wild-type haploid strain of *Saccharomyces cerevisiae* grown in batch culture over a wide range of doubling times (τ) have been measured using microscopic measurements and a particle size analyzer. Mean size increased over a 2.5-fold range with increasing growth rate (from τ = 450 min to τ = 75 min). Mean size is principally a function of growth rate and not of a particular carbon source. The duration of the budded phase increased at slow growth rates according to the empirical equation, budded phase = $0.5\tau + 27$ (all in minutes). Using a recent model of the cell cycle in which division is thought to be asymmetric, equations have been derived for mean cell age and mean cell volume. The data are consistent with the notion that initiation of the cell cycle occurs at "start" after attainment of a critical cell size, and this size is dependent on growth rate, being, at slow growth rates, 63% of the volume of fast growth rates. Previous reports are reanalyzed in the light of the unequal division model and associated population equations.

In at least some bacteria it is well established that there is a simple relationship between growth rate and cell size, where dry weight, total protein, or volume is used as a measure of size (15, 20). This is thought to be a natural consequence of two "rules" that govern the cell cycle, namely, that both mass per chromosome origin at the initiation of DNA synthesis (5) and the time from initiation to division (C + D period) are constant over a wide range of growth rates (14).

Similar studies on eucaryotes are rare, but those on *Schizosaccharomyces pombe* have revealed a more complex situation with size controls over both the initiation of DNA synthesis (19) and nuclear division (6). Studies on *Saccharomyces cerevisiae*, in either glucose-limited (16, 18) or ammonium-limited (16) chemostat culture, clearly show that fast-growing cells are bigger than slower-growing cells. Combined with the observation of apparent constancy of the budded phase (equivalent to S + G2 + M) in glucose-limited chemostat culture (9, 22) and batch culture (10), there is an obvious analogy with the results for *Escherichia coli*. Some recent shift-up experiments demonstrating rate maintenance have tended to reinforce this view (4). However, there are four points that suggest this is an oversimplified interpretation. First, it has been observed that there are differences in results when the same strains are grown at the same growth rates in batch and chemostat cultures (1). Second, autoradiographic data show that although the S phase is relatively invariant, other parts of the budded phase are elongated at slower growth rates (2). Third, altering the growth rate by cycloheximide treatment (8) or by nitrogen limitation (C. Rivin, personal communication) leads to an increase in length of the budded phase. Fourth, there is the important discovery that budding yeast has an asymmetric cell cycle, mother cells cycling faster than daughter cells at all growth rates except maximal (8, 21). The evidence suggests that at a particular growth rate yeast cells have to attain a critical cell size (8–10, 12) before initiating the cell cycle at "start" (7), bud emergence occurring shortly afterwards. Since mother volume remains relatively unaltered during growth of the bud (11), after cell separation, mothers have already attained the critical size and can initiate a further cycle immediately from start, whereas daughters may have to grow first before entering a second cycle.

We have investigated the relationships between mean cell size, duration of the budded phase, and growth rate, using a wide range of different media and growth rates, and interpreted the data in the light of the asymmetric model of the yeast cell cycle.

MATERIALS AND METHODS

Organism. A wild-type haploid strain of *S. cerevisiae* was obtained from C. F. Roberts, Genetics Department, University of Leicester, Leicester, U.K.

Media. Six different basal media were used together

with different carbon sources. The compositions of the basal media per liter were as follows: YEP—yeast extract, 10 g; peptone, 20 g; carbon source, 20 g; EMM—NH_4Cl, 5 g; NaH_2PO_4, 300 mg; sodium acetate, 1 g; KCl, 1 g; $MgCl_2$, 500 mg; Na_2SO_4, 100 mg; $CaCl_2$, 100 mg; inositol, 10 mg; nicotinic acid, 10 mg; calcium pantothenate, 1 mg; citric acid, 1 mg; boric acid, 500 ng; $MnSO_4 \cdot H_2O$, 400 ng; $ZnSO_4 \cdot 7H_2O$, 400 ng; $FeSO_4 \cdot 7H_2O$, 200 ng; Na_2MoO_4, 100 ng; $CuSO_4 \cdot 5H_2O$, 40 ng; biotin, 10 ng; carbon source, 20 g; MM1—yeast nitrogen base without amino acids (Difco), 6.7 g; sugar, 40 g; MM2—yeast nitrogen base with amino acids, 6.7 g; sugar, 40 g: MM3—yeast nitrogen base without amino acids, 6.7 g; yeast estract, 1 g, carbon source, 10 g; potassium hydrogen phthalate, 10.2 g; MM4-yeast nitrogen base without amino acids, 6.7 g; carbon source, 10 g; potassium hydrogen phthalate, 10.2 g. Carbon sources used and overall composition of the media are given in Table 1. Some media were sterilized by autoclaving, and all were also filter sterilized to produce particle-free media.

Growth conditions. All experiments were performed in a shaking water bath at 30°C containing 250-ml Erlenmeyer flasks with 100 ml of medium. Growth was monitored as cell number, using a particle counter (see below), and measurements were only taken when cells were in exponential growth both before and after the period of sampling (at least two doubling times). Cells were fixed in 4% formaldehyde, briefly sonicated to separate clumps and divided cells, and measured the same day.

Cell counts and volume determination. Cell counts and volume determination were done with an Electrozone/Celloscope model 111 LTS (Particle Data Inc., Elmhurst, Ill.), using a 60-μm orifice and flow rate of 0.42 cm/s at cell densities low enough to avoid coincident counting. Cell volume distributions were obtained using a Nuclear Data 1100 analyzer system (Nuclear Data Inc., Palatine, Ill.) coupled to a Hewlett-Packard XY plotter (Hewlett-Packard Inc., Pasadena, Calif.), and mean cell volumes were obtained from the peak of the normal distribution of volumes (on a log scale). Six samples were taken from each experiment, and the machine was calibrated using standard latex spheres (2.03 and 5.7 μm in diameter) obtained from Dow Chemical Co.

Direct microscopic measurements of more than 200 cells for some samples and assumption of the shape of the cell being a prolate spheroid gave exactly the same mean cell volume. The proportion of budded cells was obtained from counting about 1,000 cells.

RESULTS

Mean cell volume. The data in Table 1 clearly show that mean size increases with growth rate, especially at the faster growth rates. There is a consistent relationship between cell size and growth rate for any one medium even when doubling times vary due to differences in separate batches of media. Occasionally, the same medium and growth rate results in a substantial variation in mean cell size (e.g., MM1 plus fructose). There is no obvious pattern of

TABLE 1. *Population doubling time, percent budded cells, and mean cell volume for different batch culture media*

Medium	Doubling time (τ) (min)	Mean vol $\bar{V}$ (μm^3)	% budded cells
YEP + fructose	71.0	42.4	73.4
	73.4	42.1	77.4
	73.6	41.3	74.0
	76.5	35.3	71.9
	77.2	40.8	74.4
	78.0	40.7	70.6
	91.2	28.6	74.4
YEP + glucose	75.0	35.7	80.6
	75.6	38.8	81.4
	76.8	34.3	NM[a]
	76.9	40.6	88.6
	80.8	39.4	82.0
	83.6	31.1	72.7
	84.2	36.6	75.5
YEP + sucrose	76.8	27.8	73.0
	77.9	41.1	81.6
	79.4	42.5	78.9
	84.0	26.0	71.0
YEP + mannose	81.6	28.2	72.4
YEP + maltose	105.0	22.8	65.5
	109.5	20.8	57.8
	115.0	21.8	60.6
	165.0	19.4	40.3
YEP + raffinose	109.2	18.0	67.3
YEP + sorbitol	139.5	15.9	64.5
YEP + gluconate	143.4	25.8	74.4
YEP + galactose	148.5	21.1	53.4
	153.8	21.3	62.3
	166.5	22.2	NM
YEP + glycerol	159.0	22.8	54.2
	161.4	21.9	57.4
	165.0	27.6	57.4
YEP + mannitol	193.2	20.0	68.6
YEP + trehalose	194.2	17.8	68.8
MM1 + glucose	117.6	25.5	62.7
MM1 + maltose	120.0	16.6	62.8
	120.6	18.9	69.3
MM1 + fructose	123.0	16.4	69.0
	126.8	29.2	64.7
MM1 + glycerol	245.0	NM	36.7
	276.0	17.0	NM
	308.0	16.3	NM
	345.0	17.4	NM
MM2 + fructose	90.6	34.0	75.2
MM2 + glucose	98.2	25.9	69.7
MM3 + acetate	180.0	18.1	64.2
	204.0	25.0	64.8
MM3 + citric acid	435.0	16.9	50.8
MM4 + acetate	381.0	19.5	46.0
EMM + galactose	268.0	23.5	NM
	276.0	22.2	51.5
EMM + acetate + phthalate	432.0	19.2	41.0
	477.0	18.3	47.8

[a] NM, Not measured.

dependency of cell size on a particular medium, but at intermediate growth rates there is considerable variation in size for different media. This is illustrated in Fig. 1 where a convenient plot is obtained with $\bar{V}$ against $1/\tau$. The largest cells are 2.67 times the smallest cells. There is a suggestion of a minimum size at about 16 μm^3 at slower growth rates. The best fit to the data is an empirically fitted curve of increasing slope at faster growth rates.

Duration of budded phase. The percentage of budded cells decreases as growth rate decreases (Table 1), as has been observed before (7, 22), and it is possible to calculate the duration of the budded phase using equation 5 (see Appendix). (Note that this equation is equally valid for symmetrically or asymmetrically dividing yeast populations.) A plot of the duration of the budded phase against doubling time is shown in Fig. 2. There is a clear linear relationship between the two according to the empirical equation $B = 0.5\tau + 27$, where B is the duration of the budded phase; all values are in minutes.

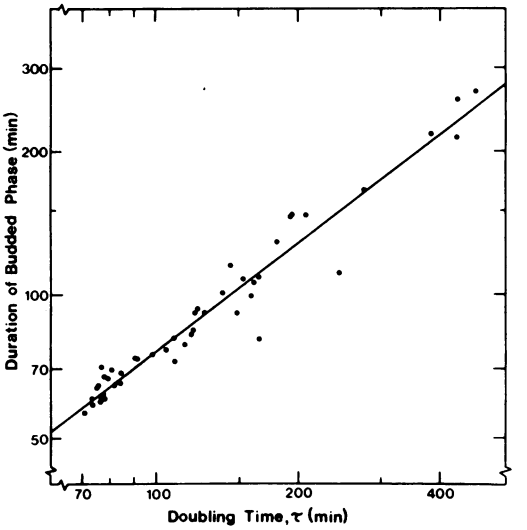

FIG. 2. *Duration of the budded phase as a function of growth rate plotted on a double-log scale. The budded phase was calculated from Table 1 and equation 5 (see Appendix). The straight line was fitted by linear regression ($r^2 = 0.95$).*

DISCUSSION

The increase in mean cell size at faster growth rates agrees with previous evidence from both chemostat (10, 16, 18) and batch (10) cultures. We also found that the budded phase varied with growth rate according to the empirical relationship $B = 0.5\tau + 27$ min. This compares with a value of $B = 0.17\tau + 87.4$ min obtained by Hartwell and Unger (8) using a variety of strains at 23°C. The cause of the difference may be related to the method of manipulation of the growth rate, media composition in the former case and mutation and cycloheximide addition in the latter case. However, analysis of other data in the literature gives values of from 0.13τ + 110 min to 0.6τ + 39 min (A. E. Wheals, manuscript in preparation), and there is no obvious correlation between methodology, strain, and temperature on the one hand and result on the other.

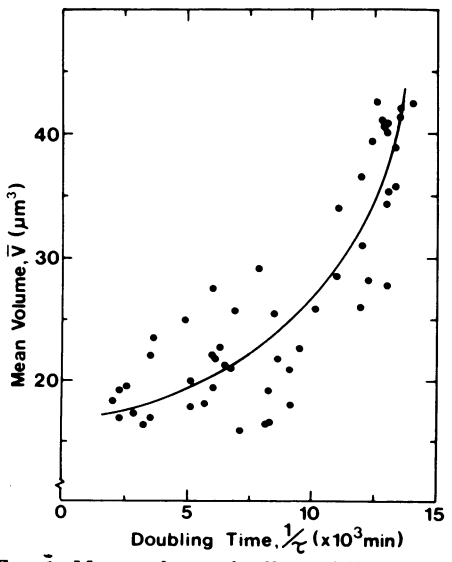

FIG. 1. *Mean volume of cells at different growth rates. Volumes were measured with an electronic particle counter, and growth rate was determined by increase in particle number during exponential growth over two doubling times (τ).*

To interpret our data in quantitative terms it is necessary to see how population variables alter in response to growth rate in the light of the asymmetric model of the yeast cell cycle (8, 21). We have used Hartwell and Unger's terminology and derived a number of relationships (see Appendix). We explicitly give the equation for the duration of the budded phase in terms of the doubling time and fraction of budded cells (equation 5) as

$$B = \frac{\ln (F_B + 1)}{\ln 2} \cdot \tau$$

even though this has appeared before in other forms (2) since it has previously been erroneously presented (22).

The mean cell age is given (equation 6) by

$$\bar{t} = D + P - \frac{1}{\alpha}$$

and hence at all growth rates below maximum it is an average of two subpopulations cycling at different rates.

To calculate the mean cell volume, we have assumed (as have Hartwell and Unger) that growth in size was exponential throughout the cell cycle. Such an assumption seems reasonable in the light of the recent evidence that practically all RNA and protein species are synthesized at an exponentially increasing rate throughout the cycle (S. Elliott and C. McLaughlin, personal communication). Mean volume is then given in terms of a reference volume, V_p, the volume at "start" (equation 9), by

$$\bar{V} = V_p \propto (De^{\propto P} - D + P)$$

Using this equation we have explored how mean cell volume varies with growth rate. We have good estimates of B and τ (and hence $\propto$), but in order to fix P (and thus calculate D) the magnitude of P-B (the time from the initiation of the cycle at start to the onset of budding) has to be obtained. $D = P = \tau$ at the maximum doubling time, which in this case is about 75 min. The duration of the budded phase at this doubling time is 64.5 min (from $B = 0.5\tau + 27$) and hence P-B is 10.5 min, similar to the value of 12 min obtained by Hartwell and Unger at their fastest growth rate of 150 min. They found that P-B increased at slower growth rates. Since their experiments were performed on a different strain at 23°C, we have not used their specific empirical relationship but have calculated $\bar{V}$ twice, on the assumption of constancy and of steady increase for comparison (Fig. 3, curves A and B). In neither case is there more than a 50% increase in mean cell size, whereas a 250% increase was observed. In addition, we have calculated $\bar{V}$ assuming constancy of the duration of the budded phase (Fig. 3, curve C), contrary to our own observations (Fig. 2). In this case we obtain the correct magnitude of the observed change in mean volume, but the curve is of the wrong shape, being convex rather than concave.

There are four ways in which a size-regulated initiation of division can yield a greater than twofold change in mean volume: if the cell cycles overlap (as in bacteria); if the cell cycle is asymmetric; if the time from initiation to division is large or variable; if the initiation size varies with growth rate. There is no evidence for the first alternative, but there is good evidence for the second (8–10) and third (at least in this case). However, asymmetry and variable budding time alone are insufficient to produce the observed relationship (Fig. 1 and 3). We conclude that it is also necessary to suggest that there is a growth rate-modulated size control over start. Calculating back from the curves in Fig. 1 and 2, we deduce that V_p varies smoothly from about 17 μm^3 at slow growth rates to 28.5 μm^3 at $\tau = 75$ min, with most of the change occurring at growth rates faster than $\tau = 100$ min (Fig. 4). (If we additionally assume that P-B varies with growth rate [see reference 8; Fig. 5] as shown in Fig. 3, curve B, then the shape of curve B in Fig. 4 remains the same, but the plateau at slow growth rates occurs at about 18.5 μm^3.) Qualitatively, similar conclusions have been obtained by B. L. A. Carter (personal communication), albeit on strains growing at 23°C, by direct measurement of cells with incipient buds (close to V_p) isolated from populations growing at different growth rates. Both of these results are difficult to reconcile with those of Adams (1), who not only found mean size to be largely invariant with growth rate but also that diploids at slow growth rates were the same size as haploids (cf. 3).

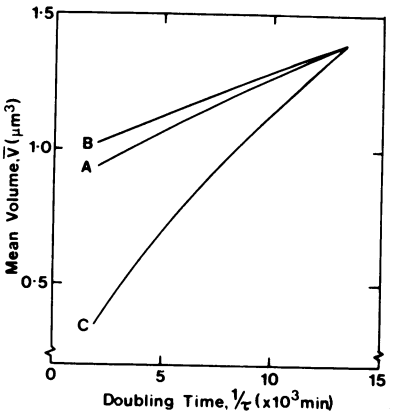

FIG. 3. *Mean volume of cells growing at different growth rates. The three curves were calculated from equation 9, assuming (A), $B = 0.5\tau + 27$ min, $\tau_{max} = 75$ min, P to B constant at 10.5 min, $V_p = 1$ arbitrary unit; (B) as (A) except P to B is variable, doubling in duration from 10.5 min at $\tau = 75$ min for every doubling in τ; (C) as (A) except P and B both constant, totaling 64.5 min.*

It can also be seen from Fig. 4 that both size at cell separation and size of daughters at birth vary with growth rate. In terms of the control of cell division, there are two ways in which the data can be interpreted. (i) Division is initiated at a critical size, V_p, and occurs a fixed time, P, later, both the size and the time being modulated by growth rate (sizer plus timer model [6]). (ii) Both budding and division are initiated at particular critical sizes, the sizes both being modulated by growth rate. Such a situation pertains to *S. pombe* (6).

Two interesting consequences arise from the

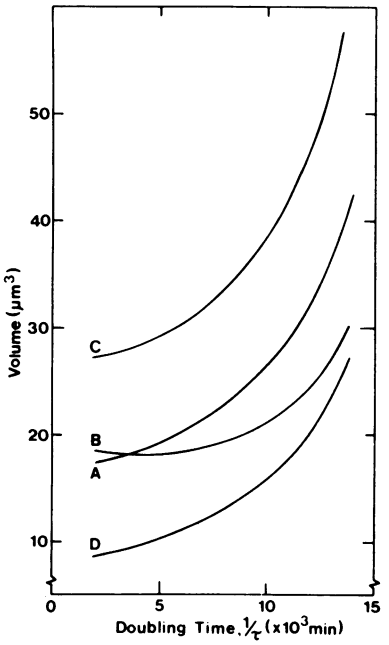

FIG. 4. *Cell volume at particular points in the cell cycle as a function of growth rate. Curve (A) is redrawn from Fig. 1 and represents mean cell volume of the population. Curve (B) is the volume of a cell at "start," V_p, and has been calculated using $\tau_{max} = 75$ min, $B = 0.5\tau + 27$ min, P to B constant at 10.5 min, and altering V_p as a function of growth rate to give the best fit to curve (A), using equation 9. Curve (C) is the volume of a mother and daughter cell combined at the time of cell separation, using the values for curve (B) and equation 8. Curve (D) is the volume of a daughter cell at birth, using the values in curve (B) and equation 8.*

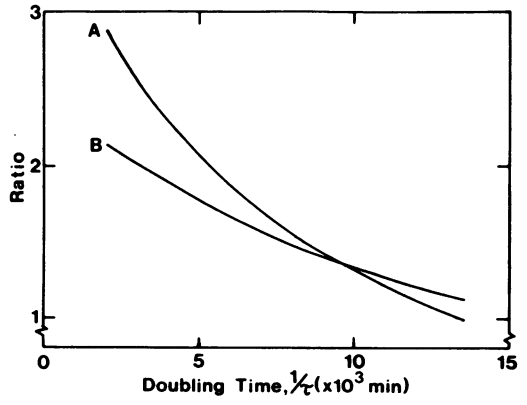

FIG. 5. *Ratio of mother to daughter parameters as a function of growth rate. Both curves were drawn using values for curve (B) in Fig. 4. Curve (A) represents the ratio of cycle times of mothers to daughters, using equation 1. Curve (B) represents the ratio of birth volume of mothers to daughters, using equations 8 and 9.*

asymmetry of division in yeast. As the growth rate decreases, the ratio between mothers and daughters of both volume at birth and cycle time rises dramatically so that at $\tau = 500$, mothers are about twice as big as daughters at birth, and the cycle time of daughters is about three times as long (Fig. 5). At fast growth rates the ratio approaches one to one, and this sets the upper limit to balanced growth. If the cells were growing in mass or volume with a doubling time faster than this, the rate of division would be unable to keep up and abnormally large cells could result. Interestingly, such a phenomenon has been seen in *Candida utilis* in two-stage fermentors where mass doubling times have been less than 60 min (23).

Finally, it is worth pointing out that the derivation of population cell cycle kinetics for budding yeast in this paper and previously (8, 21) should provide the framework for similar analyses on other asymmetrically dividing cells such as budding bacteria (24) and blue-green algae (cyanobacteria) (17).

APPENDIX

Hartwell and Unger (8) have defined the age distribution of cells undergoing unequal division. This distribution is represented diagrammatically in Fig. 6 which is redrawn from reference 8 with the addition of a point, B, the initiation of budding. The age, t, of a cell at a particular point in the cell cycle is given in terms of the length of time which that cell will take to reach division and is in units of τ, the population doubling time. Thus, at division a cell will be of age 0; D is the daughter cell cycle time, and P is the parent cell cycle time.

The relationship between D and P is given by the expression

$$e^{\alpha D}[1 - e^{-\alpha P}] = 1 \qquad (1)$$

The age distribution is given by

$$\begin{aligned} \phi(t) &= \alpha e^{\alpha t} && 0 < t < P \\ \phi(t) &= \alpha e^{\alpha t}[1 - e^{-\alpha P}] && P < t \leq D \\ \phi(t) &= 0 && t = 0 \end{aligned} \qquad (2)$$

where $\phi(t)$ is the probability density function and $\alpha = \ln 2/\tau$.

The frequency of cells per unit time passing through a point, t, in the cycle is

$$N_{(t)} = N_{(0)}\, \alpha e^{\alpha t} \qquad (3)$$

where $0 \leq t < P$, and

$$N_{(t)} = N_{(0)}\, \alpha e^{\alpha t}[1 - e^{-\alpha P}] \qquad (4)$$

where $P < t \leq D$.

Duration of budded phase. Using these equations, it is possible to find out B, the budding interval,

from the fraction of budding cells observed in an asynchronous culture. This specific derivation was not explicitly described previously (8). The fraction of budded cells is found by integrating equation 3 between B and 0.

The fraction of budded cells

$$F_B = \int_0^B \alpha e^{\alpha t} dt$$

$$F_B = e^{\alpha B} - 1$$

or

$$B = \frac{\ln(F_B + 1)}{\alpha}$$

$$B = \frac{\ln(F_B + 1)}{\ln 2} \cdot \tau$$

$$B = \frac{\ln(F_B + 1)}{\ln 2} \cdot \tau \qquad (5)$$

Derivation of mean cell age. The mean cell age is found by multiplying the density function, $\phi(t)$, by the variable, t, and integrating between the appropriate limits.

The mean cell age, $\bar{t}$, is then

$$\bar{t} = \int_P^D t \cdot \alpha e^{\alpha t}(1 - e^{-\alpha P})dt + \int_0^P t \cdot \alpha e^{\alpha t} dt$$

$$= \left[t \cdot e^{\alpha t}(1 - e^{-\alpha P}) - \frac{1}{\alpha} e^{\alpha t}(1 - e^{-\alpha P}) \right]_P^D + \left[t \cdot e^{\alpha t} - \frac{1}{\alpha} e^{\alpha t} \right]_0^P$$

$$= De^{\alpha D}(1 - e^{-\alpha P}) - \frac{1}{\alpha} e^{\alpha D}(1 - e^{-\alpha P}) - Pe^{\alpha P}(1 - e^{-\alpha P}) + \frac{1}{\alpha} e^{\alpha P}(1 - e^{-\alpha P}) + Pe^{\alpha P} - \frac{1}{\alpha} e^{\alpha P} + \frac{1}{\alpha}$$

$$= D - \frac{1}{\alpha} - Pe^{\alpha P} + P + \frac{1}{\alpha} e^{\alpha P} - \frac{1}{\alpha} + Pe^{\alpha P} - \frac{1}{\alpha} e^{\alpha P} + \frac{1}{\alpha}$$

$$\bar{t} = D + P - \frac{1}{\alpha} \qquad (6)$$

If we assume that at maximum doubling time cells divide symmetrically, then we can say that at this doubling time $D = P = \tau$. The mean cell age in this case, using equation 6, is $2 - \tau/\ln 2$, which is 0.557 (see 13).

Mean cell volume. We assume that cell mass (M) increases exponentially such that at a time, t

$$M_{(t)} = M_{(0)} e^{\alpha t} \qquad (7)$$

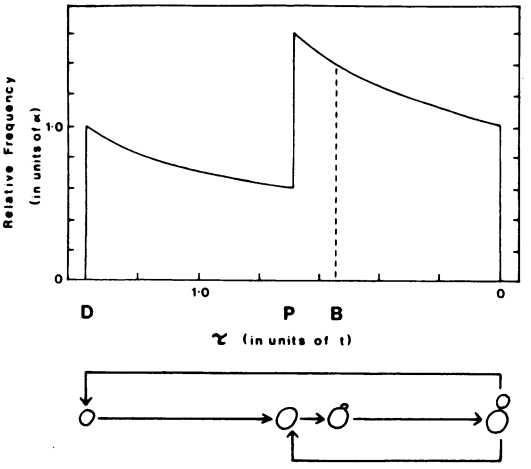

FIG. 6. *Age distribution and corresponding stage in the cell cycle of asymmetrically dividing yeast cells, redrawn from reference 8.* α *is* $\ln 2/\tau$, *D is beginning of daughter cycle, P is start of parent (mother cycle) at "start," B is start of budding phase, and 0 is time of cell separation. The corresponding mother and daughter cycles are drawn underneath.*

If the density of a cell remains relatively constant throughout the cell cycle, then we can replace M in equation 7 by V (cell volume).

Unbudded cells have to attain a minimum size at P (= start) before a cycle is initiated (8) at point P in the cycle. The volume of a cell at any point, t, in the cycle can be expressed in terms of volume at $P(V_p)$, as

$$V_{(t)} = V_{(0)} e^{\alpha(P-t)} \qquad (8)$$

The mean cell volume, $\bar{V}$, is found by multiplying the density function, $\phi(t)$, of the age distribution by the volume at a point, t, and integrating between the appropriate limits. Hence

$$\bar{V} = \int_P^D \alpha e^{\alpha t}(1 - e^{-\alpha P}) \cdot V_p e^{\alpha(P-t)} \cdot dt + \int_0^P \alpha e^{\alpha t} \cdot V_p e^{\alpha(P-t)} \cdot dt$$

$$= V_p \int_P^D \alpha e^{\alpha P}(1 - e^{-\alpha P})dt + V_p \int_0^P \alpha e^{\alpha P} dt$$

$$= V_p [t \cdot \alpha e^{\alpha P}(1 - e^{-\alpha P})]_P^D + V_p [t \cdot \alpha e^{\alpha P}]_0^P$$

$$= V_p \alpha [e^{\alpha P}(1 - e^{-\alpha P})(D - P) + Pe^{\alpha P}]$$

$$= V_p \alpha [De^{\alpha P} - D - Pe^{\alpha P} + P + Pe^{\alpha P}]$$

$$\bar{w} = V_p \alpha (De^{\alpha P} - D + P) \qquad (9)$$

In the case of a symmetrically dividing population

of cells, i.e., when $D = P = \tau$, the mean cell volume, $\bar{V}$, is $V_p \cdot 2 \ln 2 = 1.386\ V_p$ (see 13).

This assumes that all the volume increase of a parent cell in the period between P and 0 is distributed to the daughter cell.

ACKNOWLEDGMENTS

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