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Regulation of Cell Size in the Yeast *Saccharomyces cerevisiae*

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For cells of the yeast *Saccharomyces cerevisiae*, the size at initiation of budding is proportional to growth rate for rates from 0.33 to 0.23 h^{-1}. At growth rates lower than 0.23 h^{-1}, cells displayed a minimum cell size at bud initiation independent of growth rate. Regardless of growth rate, cells displayed an increase in volume each time budding was initiated. When abnormally small cells, produced by starvation for nitrogen, were placed in fresh medium containing nitrogen but with different carbon sources, they did not initiate budding until they had grown to the critical size characteristic of that medium. Moreover, when cells were shifted from a medium supporting a low growth rate and small size at bud initiation to a medium supporting a higher growth rate and larger size at bud initiation, there was a transient accumulation of cells within G1. These results suggest that yeast cells are able to initiate cell division at different cell sizes and that regulation of cell size occurs within G1.

Yeast cells in culture maintain a constant cell size through coordination between the processes of growth and cell division (3, 10). Growth does not normally occur in the absence of cell division, producing very large cells, nor does division normally continue in the absence of growth, producing very small cells. Recently, we suggested the following scheme for the coordination of growth and cell division in the yeast *Saccharomyces cerevisiae*: the completion of an essential event in the G1 phase of the cell cycle requires growth to some critical size (8, 10), and growth is always rate limiting for progression through the cell division cycle (10). We noted that under conditions of nutrient starvation small G1-arrested cells were produced. When placed in fresh medium, these cells grew to a critical size before initiating a cell division cycle. These observations suggested that, regardless of growth conditions, a cell must attain some critical size before initiation of cell division may occur. The yeast is a particularly suitable organism for such study, since the initiation of cell division or DNA synthesis is coincident with the onset of budding (15) (thus, cells within G1 are unbudded).

We have now determined if the same critical size is required for initiation of cell division by cells growing at different rates. We report that under different growth conditions yeast cells are able to initiate cell division at different cell sizes.

MATERIALS AND METHODS

Strains and growth conditions. The yeast *S. cerevisiae* was studied. Haploid cells of strain C4.2 (derived from the diploid C276, kindly provided by J. R. Pringle) were cultured at a variety of steady-state growth rates in a glucose-limited medium in a chemostat (L. H. Engineering Co., Ltd., Bucks, England) at 24°C (7). The diploid strain AG1-7 and the isogenic haploid GR2 have been described elsewhere (11). Cells were also grown in a minimal medium (yeast nitrogen base without ammonium sulfate or amino acids [YNB]; Difco Laboratories, Detroit, Mich.) and an enriched medium (YM1). Both media have been previously described (10).

Photomicroscopy and cell volume determination. Cells were collected either from chemostat samples or from batch culture by centrifugation and suspended in 1 ml of a solution of Calcofluor (American Cyanamide Co., Pearl River, N. Y.) (6). The concentration of Calcofluor used was either 2 (Table 1) or 10 (Tables 2 and 3) mg/ml in water. Stained cells were photographed with a Zeiss fluorescence microscope. Photographs were taken with either Kodak Tri X Pan film or Kodak 2475 recording film. A stage graticule (Graticule, Ltd., Kent, England) was photographed with every roll of film so that absolute cell volumes could be determined. As an alternative internal standard for cell size, photographs were taken of a mixture of yeast cells and latex beads of a uniform diameter of 3.49 μm (obtained from Coulter Electronics, Inc., Hialeah, Fla.).

Budding history (number of buds previously produced by a cell) was determined by counting the number of fluorescent bud scars on the surface of the cell. For cell volume measurements, negatives were projected onto a screen, and the lengths of the major and minor axes were determined. Cell volume was calculated by assuming the yeast to approximate a prolate spheroid. Only those cells with small buds (less than 10% of the parent cell volume) were measured.

Measurement of cell number and cell volume distribution. Cell numbers were determined with a

Coulter Counter (Coulter Electronics) as previously described (4). Median cell volume of cultures was determined with a Coulter Channelyzer.

RESULTS

Variation of cell volume at bud initiation with growth rate. To investigate the relation between growth rate and cell size at bud initiation, we examined cells grown both in chemostat and in batch cultures. Use of both techniques allowed consideration of a wider range of growth rates.

Cells of the haploid strain C4.2 were cultured at a variety of steady-state growth rates by glucose limitation in a chemostat at 24°C. Samples were removed from the chemostat at each growth rate, and the median cell volume was determined with a Coulter Channelyzer. For growth rates from 0.33 to 0.23 h^{-1} (generation times of 2.1 to 3 h), the median cell volume was proportional to the growth rate (Fig. 1). Steady-state cultures at growth rates from 0.23 to 0.077 h^{-1} (generation times of 3 to 9 h) had approximately the same median cell volume. Thus, at lower growth rates, cell volume became independent of growth rate.

The volumes of cells initiating buds were determined for cells cultured at different steady-state growth rates in the chemostat. Since under some growth conditions a cell increases in volume each time it buds, we grouped volume measurements of cells according to the budding histories of the cells to reduce volume heterogeneity. The volumes of cells of different bud scar classes initiating buds under steady-state growth conditions are shown in Table 1 and Fig. 2. At all growth rates, cells with more bud scars initiated bud formation at larger sizes than did cells with fewer bud scars. Moreover, within any bud scar class, cell volume at bud initiation was proportional to growth rate at rates greater than 0.23 h^{-1}; at lower rates, cell volume at bud initiation was independent of growth rate, suggesting a minimum cell size for bud initiation.

In similar fashion, we examined the effect of growth rate in batch culture on size at bud initiation. Growth rate was altered by the use of different carbon sources. Cells of the diploid yeast strain AG1-7 were grown for several generations by repeated subculturing. As found for chemostat-grown populations, reduced growth rates led to smaller median cell volumes (data not shown).

TABLE 1. *Effect of altered growth rate[a] on volume at bud initiation*

Growth rate (h^{-1})	Cell volume (μm^3) at bud initiation			
	0 bud scar	1 bud scar	2 bud scars	3 (or more) bud scars
0.310	40.2 ± 4.8	47.8 ± 5.0	55.4 ± 7.2	70.0 ± 17.0
0.270	38.7 ± 4.5	48.5 ± 6.0	55.2 ± 7.5	65.7 ± 9.0
0.230	30.6 ± 3.0	37.0 ± 3.0	43.6 ± 7.3	58.5 ± 14.0
0.195	28.5 ± 3.6	33.8 ± 3.8	40.7 ± 7.6	48.0 ± 11.0
0.180	27.8 ± 3.3	31.6 ± 4.5	36.9 ± 3.6	44.0 ± 14.0
0.120	29.7 ± 4.2	35.8 ± 5.0	40.9 ± 7.0	50.3 ± 9.8
0.110	28.8 ± 5.1	34.9 ± 5.4	40.8 ± 8.0	50.0 ± 19.0
0.078	29.7 ± 5.6	35.9 ± 6.1	42.9 ± 7.8	49.3 ± 3.0
0.077	31.5 ± 6.5	37.1 ± 5.6	45.7 ± 7.5	53.7 ± 9.6

[a] Altered by glucose limitation in a chemostat.

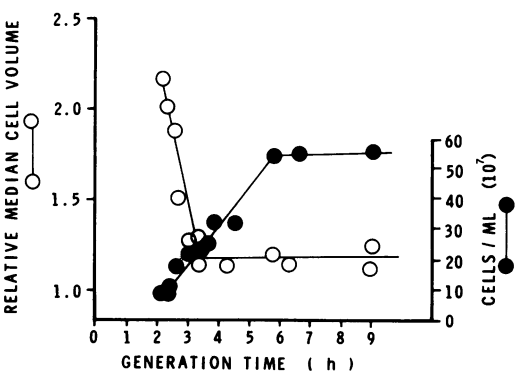

FIG. 1. *Variation of median cell volume with growth rate. The growth rate of the haploid strain C4.2 was varied by glucose limitation in a chemostat. At various steady-state growth conditions, samples were removed and the median cell volumes were determined with a Coulter Channelyzer. For the same samples, cell concentrations were also determined with a Coulter Counter. Cell concentration in the chemostat was independent of growth rate at rates less than 0.175 h^{-1} (generation time of 3.96 h) but decreased at higher growth rates.*

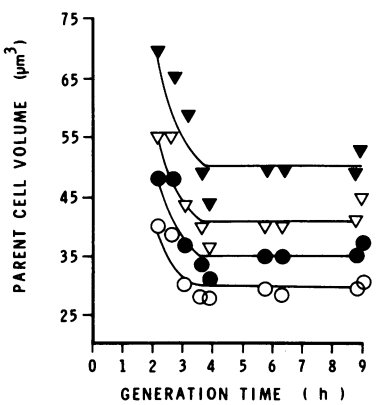

FIG. 2. *Relationship between growth rate and size at bud initiation. Growth rates of the haploid strain C4.2 were varied by glucose limitation in a chemostat. At various steady-state growth conditions, cells were removed and the volumes of cells initiating buds were determined as described in the text. Symbols: ○, 0 bud scar; ●, 1 bud scar; ▽, 2 bud scars; ▼, 3 or more bud scars.*

The sizes of cells of different budding histories at bud initiation were also determined under these different growth rate conditions. When cells of AG1-7 were grown in media containing different carbon sources, growth rates varied by 2.5-fold (Table 2). At all growth rates, cells with more bud scars initiated budding at larger sizes than did cells with fewer bud scars. Within any bud scar class, the volume at initiation of budding was proportional to growth rate. Results obtained under all growth conditions were consistent with this trend except for those from YNB medium with potassium acetate as the carbon source. In this medium, cell size was slightly larger than expected; nevertheless, these cells were still markedly smaller at initiation of budding than were cells growing in YNB medium with glucose as the carbon source.

The relationship between growth rate and cell size at initiation of budding is not a strain-specific phenomenon. Cell size at initiation of budding was proportional to growth rate for the haploid strain GR2 (Table 2), isogenic to AG1-7, and for the unrelated prototrophic diploid strain C276 (data not shown). Since chemostat and batch cultures displayed equivalent alterations in cell size with growth rate, subsequent experiments were performed solely with batch cultures.

Alteration in cell size with different nitrogen sources. Cells of the diploid strain AG1-7 were cultured for several generations by repeated subculturing in YNB with glucose as a carbon source and either proline, leucine, or ammonium sulfate as a nitrogen source. Cell size at initiation of budding again was proportional to growth rate, and each successive round of budding was accompanied by an increase in the volume of the budding cell (Table 3).

Effect of medium on size at initiation of budding of abnormally small cells. A population of cells of AG1-7 starved for nitrogen consists of both normal-sized and abnormally small cells (Fig. 1 of reference 9). A homogeneous population of these small cells was isolated by centrifugation through a linear density gradient of Ludox (10). The small cells isolated from this gradient were smaller than any of the budding cells found under any growth condition described above (average cell volume was approximately 10 μm^3 and for more than 90% of the isolated population was less than 20 μm^3). These small cells, all of the zero-bud-scar class, were then placed in different media to initiate growth. Cell sizes at initiation of budding were dependent on the medium and were similar to the sizes of budding cells in long-term growth experiments (Table 2). Small cells placed in YNB medium containing glucose or YM1 medium containing glycerol or acetate as a carbon source grew to 68.5 ± 17.3, 43.0 ± 12.1, or 43.7 ± 8.7 μm^3, respectively, before initiating a bud. Thus, the different cell sizes at initiation of budding observed in different media are not the result of an equilibrium between mass increase and cell cycle-specific events, but they represent an al-

TABLE 2. *Effect of carbon source on growth rate and volume at bud initiation*

Strain	Medium[a]	Growth rate (h^{-1})	Cell volume (μm^3) at bud initiation		
			0 bud scar	1 bud scar	2 bud scars
AG1-7	YM1 + 2% glucose	0.33	70.0 ± 14.7	81.1 ± 17.8	95.6 ± 17.5
	YNB + 2% glucose	0.31	64.6 ± 13.3	78.6 ± 12.5	94.2 ± 14.8
	YM1 + 3% acetate	0.19	41.4 ± 4.1	52.0 ± 7.2	76.0 ± 12.8
	YM1 + 2% glycerol	0.16	39.1 ± 7.0	44.9 ± 10.9	52.1 ± 10.9
	YNB + 3% acetate	0.12	49.8 ± 9.2	60.7 ± 12.0	76.0 ± 10.7
GR2	YM1 + 2% glucose	0.29	37.5 ± 10.6	45.4 ± 5.6	66.8 ± 9.1
	YNB + 2% glucose	0.23	41.7 ± 8.5	47.2 ± 14.7	65.0 ± 11.9
	YM1 + 2% glycerol	0.10	29.7 ± 5.0	37.5 ± 6.5	48.8 ± 10.8

[a] Media were initially made without carbon sources.

TABLE 3. *Effect of nitrogen source on growth rate of strain AG1-7 and volume at bud initiation*

Medium[a]	Growth rate (h^{-1})	Cell volume (μm^3) at bud initiation		
		0 bud scar	1 bud scar	2 bud scars
YNB + $(NH_4)_2SO_4$[b]	0.31	64.6 ± 13.3	78.6 ± 12.5	94.2 ± 14.8
YNB + proline (100 μg/ml)	0.22	57.3 ± 13.0	63.9 ± 15.2	92.0 ± 18.3
YNB + leucine (100 μg/ml)	0.20	46.0 ± 9.9	53.3 ± 11.5	68.1 ± 16.2

[a] Media were made initially without nitrogen sources.
[b] Same sample as listed in Table 2 (YNB + 2% glucose).

teration in the critical size required by a cell to initiate a division cycle.

Effect of shiftup on the ability to initiate a bud. If cell size at bud initiation is controlled, then nutrient shift experiments should give transient alterations in the proportion of cells with buds. A population growing at a low growth rate able to initiate buds at a small cell volume should display a transient increase in the number of cells without buds when shifted to conditions supporting faster growth and requiring a larger cell volume for initiation of budding as unbudded cells grow to the newer, larger critical size. Figure 3 shows the results of a shift of strain AG1-7 from minimal medium with acetate as the carbon source to the same minimal medium with glucose as the carbon source. As previously shown (1a), this shiftup resulted in a period of rate maintenance, during which the rate of cell division was not altered, followed by an abrupt increase in the rate of cell division. During the period of rate maintenance, the population contained a larger proportion of cells without buds. Thus, under conditions supporting faster growth and requiring a larger cell volume for bud initiation, cells previously initiating buds at a small cell size will accumulate in G1 and grow to the new critical size before initiating a bud.

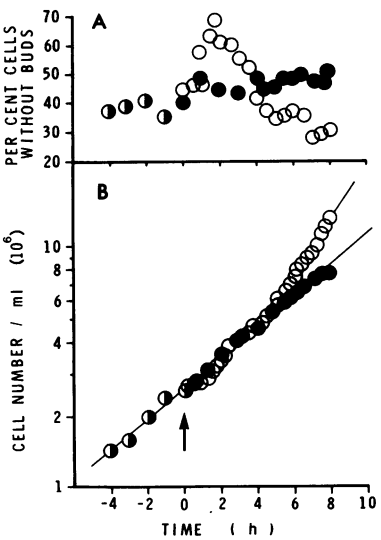

FIG. 3. *Effect of shiftup on the proportion of cells without buds. A culture of strain AG1-7 cells in YNB medium with acetate as the sole carbon source was split in half, and to one half was added glucose to a final concentration of 2%. (A) Proportion of cells without buds; (B) cell concentration. Symbols: ●, cells using acetate as sole carbon source; ○, cells using glucose as a carbon source.*

DISCUSSION

For initiation of cell division, cells of the yeast *S. cerevisiae* require growth to some critical size (8, 10). Here, we report three major findings about the relationship between this critical size and growth rate.

First, the critical size for initiation of budding is a function of growth rate and is dependent on the growth medium. For growth rates from 0.33 to 0.23 h^{-1} (generation times of 2.1 to 3 h), the size of cells initiating buds was proportional to the growth rate. Variation of cell size at bud initiation with different growth media was neither strain specific nor characteristic solely of diploid or haploid cells. In addition, this relation between growth rate and cell volume was observed both in batch cultures with different carbon and nitrogen sources and in continuous cultures. The variation in cell size at budding is a regulated response of cells to environmental differences, since abnormally small cells generated by nitrogen starvation initiated budding only after growth to the critical size characteristic of the medium. Moreover, upon shift from medium supporting a low growth rate to medium supporting a higher growth rate and requiring a larger critical size for budding, cells were transiently arrested in G1. This transient arrest probably reflects the additional growth required for cells to attain the critical size characteristic of the new medium. Thus, critical size required for initiation of cell division is stringently regulated within G1.

Second, size at bud initiation reaches a lower limit at growth rates of less than 0.23 h^{-1}. Thus, a minimum cell size is required for the initiation of cell division.

Third, in agreement with others (5, 12, 14), our results indicate that the critical size for subsequent bud initiation increases each time a cell initiates a bud. This increase in cell volume between successive rounds of bud initiation was observed at all growth rates examined. Our observation that upon shiftup cells transiently arrest in G1 suggests that such increases in size occur during the unbudded interval of the cell cycle.

Reduction of growth rate by alteration of the nitrogen source was accompanied by a reduction in parent cell size (Table 3). McMurrough and Rose (13) reported that ammonia limitation reduced the average cell volume. They did not distinguish between unbudded cells and cells that had initiated a bud, but their results are consistent with our findings.

Recently, Adams (1) studied the relationship between cell volume at initiation of budding and growth rate. In contrast to our findings, he did

not conclude that significant variation occurred in cell size at initiation of budding at different growth rates. With one notable exception, that of glucose limitation, cell volume at initiation was constant in both haploid and diploid cells. We feel that our different conclusions may stem from the methods used for measuring cell volume. In this report, we stained cells for bud scars before determining cell volume. This procedure not only allowed us to group cells by cell age and thereby reduce volume heterogeneity but also caused the outlines of the cells to be considerably sharpened compared with those of unstained cells viewed in the phase-contrast microscope. Measurements we made on unstained cells photographed under phase-contrast microscopy also failed to show significant alterations in cell size at bud initiation for different growth rates (G. C. Johnston, unpublished data). Thus, our procedure for determining cell volume may be responsible for the different conclusions that we reach.

Our results are similar to those presented by Fantes and Nurse (2), who used the fission yeast *Schizosaccharomyces pombe*. They reported that, unlike *S. cerevisiae*, cells of *S. pombe* required attainment of a certain cell size to complete cytokinesis and that cell size was normally unrelated to initiation of the cell division cycle. They noted that at reduced growth rates the sizes of dividing cells were reduced, and they termed this phenomenon "nutrient modulation." We suggest here that some sort of modulation may also be a characteristic of the cell cycle of *S. cerevisiae*. It it also apparent from our studies that cells of *S. cerevisiae* modulate size at initiation of the cell division cycle (10) and not at division.

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