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Cell Size and Budding During Starvation of the Yeast *Saccharomyces cerevisiae*

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When starved for nitrogen, cells of the yeast *Saccharomyces cerevisiae* produced abnormally small cells. Nonetheless, during starvation, only cells of a size characteristic of growing cells were capable of initiating a bud. Even when growth was severely limited, some event(s) in G1 required growth to a critical size for completion.

Cell growth is normally coordinated with events in the deoxyribonucleic acid division cycle such that cells in culture are a characteristic size (3). We recently examined the coordination of growth and division in cells of the yeast *Saccharomyces cerevisiae* (6) and concluded that growth to some critical size was essential for completion of some event(s) in the G1 portion of the cell cycle. Our conclusion was based on the growth behavior of abnormally small cells. When diploid cells of strain AG1-7 were starved for nitrogen, they increased threefold in number and eventually arrested division in G1 as cells without buds. The cell division that occurred during starvation was accompanied by a negligible accumulation of protein and ribonucleic acid. Many newly produced daughter cells were abnormally small compared with cells in a growing population (Fig. 1). As noted previously (6), these small cells, when replaced in medium containing nitrogen, required growth to some critical size before initiation of budding and deoxyribonucleic acid synthesis (unpublished data). Although growth was required for budding in nitrogen-replete medium, the small cells formed during starvation for nitrogen may have arisen from large cells (i.e., cells of critical size) producing abnormally small daughters or, alternatively, from cells initiating buds at a size smaller than that characteristic of cells in a growing population. That is, a requirement for growth to a critical size may only be a function of outgrowth from the G1-arrested state. To determine whether new, small cells were able to form buds, the sizes at which "young" cells initiated cell division and budding in nitrogen-free and nitrogen-replete media were determined.

Cell age was determined from cell morphology. In yeast, each new budding cycle is marked by the production of a new bud scar (1, 2, 4); the budding history of a cell may be determined from the number of bud scars on its surface. Thus, newly budded cells have no bud scars and after one budding cycle have one bud scar.

Cells were stained for bud scars (5; 0.1% Calcofluor; B. Sloat and J. Pringle, personal

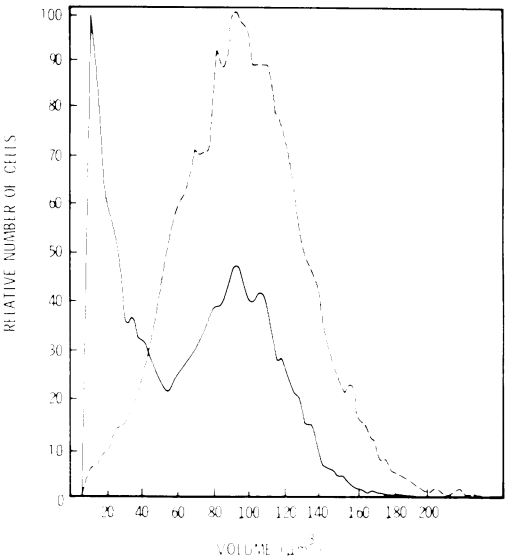

FIG. 1. *Volume distributions of cells from nitrogen-starved and exponentially growing cultures. A Coulter Channelyzer (Coulter Electronics Inc., Hialeah, Fla.) was used to obtain volume distributions of AG1-7 cells growing exponentially at 23°C in yeast nitrogen base (6) (– –) and of AG1-7 cells 9 h after transfer to nitrogen-free medium (——). The total number of cells in the starved population and the proportion of cells in the small nitrogen-starved population were determined directly with the Channelyzer. At least 50% of the total nitrogen-starved population consisted of cells of less than 50 μm³.*

communication), and the volumes of budding cells with one or no bud scar were calculated from photomicrographs (6). The size range of young cells initiating division (producing a bud) in nitrogen-free medium was characteristic of that in an exponentially growing population in the same medium supplemented with $(NH_4)_2SO_4$ (Table 1). These results suggest that, even under conditions of acutely limited growth, attaining critical size is still a prerequisite for the initiation of budding.

During nitrogen starvation, cell number increased threefold, and many cells in the final nitrogen-starved population were small (Fig. 1) and unable to bud. Thus, many large cells in the population completed two rounds of division and produced two small buds. During starvation we did note some budding cells also with completed, but not yet separated, small daughter cells. These observations indicate that small daughter cells did not arise merely from completion of divisions initiated before starvation.

These data underscore our previous conclusions on the mechanism for coordinating growth and division (6). That is, some event in G1 requires for its completion growth to a critical size even under conditions under which growth is severely limited.

TABLE 1. *Volume of cells initiating buds in medium with and without a nitrogen source*

Time (h)	Avg vol (μm^3) of young cells with small buds[a]	
	+ Nitrogen	− Nitrogen
0	72.7 ± 10.1[b]	72.7 ± 10.1
1	80.7 ± 11.7	62.5 ± 11.0
2	68.3 ± 12.0	71.2 ± 15.8
3	62.4 ± 7.3	73.8 ± 12.8
4	57.0 ± 9.8	75.5 ± 12.3
5	78.3 ± 13.3	85.9 ± 20.0
6	59.0 ± 9.2	62.1 ± 18.3
Avg	68.3 ± 9.3	71.5 ± 8.2

[a] Cell volume was calculated from photomicrographs (at least 30 cells per sample); only cells with 0 or 1 bud scar were considered. The presence or absence of bud scars was determined by focusing on both the upper and lower surface of stained cells before photographing. Data are presented as mean ± standard deviation. A paired-difference test was applied to values at each time and at 95% confidence limits; the volumes were not significantly different.

[b] The same sample of cells was used to determine the initial size of the two populations.

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