MIDCYCLE DOUBLING OF UPTAKE RATES OF ADENINE AND SERINE IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT Rates of uptake of serine and of adenine were measured as a function of cell size, and therefore age, in asynchronous, exponential phase cultures of diploid *Saccharomyces cerevisiae* strain Y55. In both cases, uptake rates were constant during the initial third of the cell cycle and doubled during the S period in the middle part of the cycle to a constant value during the final third. Cell size and age at mid-step doubling were indistinguishable for serine and adenine uptake, and occurred during the period of DNA synthesis. The results extend an earlier hypothesis of constancy of cell growth rates (mass accumulation rates) and rates of uptake of all or almost all compounds into cells in exponential phase growth to one of piecewise constancy, with an abrupt doubling of growth and uptake rates during DNA synthesis.

INTRODUCTION

In exponentially growing cultures cell mass appears to accumulate at a constant rate (linear growth) during most of the cell cycle, when growth is dependent only upon transport of compounds of low molecular weight (1). Linear or piecewise linear increase has been observed for mass increase in single cells of the fission yeast *Schizosaccharomyces pombe* (2), mass increase in the budding yeast *Saccharomyces cerevisiae* (3), and cell volume increase in *Tetrahymena pyriformis* (4). Linear increase is also supported by measurements of average or total cell length, or volume, in synchronous cultures of three different bacterial species: *Escherichia coli* (5), *Bacillus subtilis* (6), and *Salmonella typhimurium* (7). Growth rates in protozoa growing by ingestion of bacteria, however, were not constant (8, 9).

Nevertheless, measurements of cell length or volume often fail to distinguish between possible alternative growth patterns. Measurements of individual cells usually lack sufficient precision, and variability in cell length or size may well conceal true growth patterns even in synchronous cultures. Fortunately, the hypothesis of linear growth can be tested in another, much more sensitive manner.

During linear cell growth the rate of increase of cell mass must be constant, and this constancy in turn requires constancy of the net rate of uptake of all of the materials that enter the cell. It has been proposed that rates of uptake are correspondingly constant for every or almost every compound transported into the cell (1, 5, 10). In support, constant rates of uptake have been observed during most of the *E. coli* cycle for a

variety of compounds (10, 11) and constant rates of uptake of glucose and leucine were observed over almost all of the *S. pombe* cell cycle (12).

Recently, from measurements of cell length and nuclear number in *B. subtilis*, Sargent (6) concluded that growth rates, while initially constant, doubled at nuclear segregation rather than at cell division. This possible correlation had been concealed in most earlier experiments because termination of chromosome replication usually occurs near the end of the cell cycle. The experiments described below were designed to test the possibility that uptake rates might double during midcycle in cells in which DNA synthesis occurs during midcycle. The experiments were carried out with *S. cerevisiae* because this yeast is known to undergo chromosome synthesis during midcycle (see ref. 13, for example) and should therefore provide an unambiguous distinction between correlation with chromosome synthesis or with cell division.

METHODS

Strain and Culture Conditions

Saccharomyces cerevisiae strain Y55 was selected because cell clumps were dispersed to individual cells by rapid swirling or "vortexing" in test tubes (Vortex-Genie, Scientific Industries, Inc., Bohemia, N.Y.). Cells were first inoculated into a complex medium, YPD: 1% yeast extract (Difco Laboratories, Detroit, Mich.), 2% peptone (Difco), and 2% glucose. Cultures were maintained at 30°C. After several hours, these cultures were in the exponential growth phase with a doubling time of about 75 min. Cells from these cultures were used as inocula (less than 0.8% of total volume) for 100-ml cultures grown overnight on a reciprocating shaker in synthetic medium, YNB: 6.7 g of yeast nitrogen base (Difco) and 2 g of glucose, in 1 liter of water.

Cell Concentrations and Sizing

Cell densities were monitored by optical density of the cultures at 600 nm with a Zeiss PMQ II spectrophotometer (Carl Zeiss, Inc., New York), or alternatively by the use of a modified Coulter counter multichannel-analyzer system (14) (Coulter Electronics Inc., Hialeah, Fla.) for measurements of cell counts and volume distributions. The diameter of the counting aperture was 47 μm. For counting and sizing cells, samples were appropriately diluted in the YNB medium. The counter was calibrated for particle number by comparison with plate counts of viable cells. The analyzer system was calibrated for particle volume by measuring the size distribution of a suspension of polyvinyltoluene latex spheres (Dow Chemical Co., Midland, Mich.) with a mean diameter of 2.69 μm. The volumes of single, double, and triple particles passing through the aperture provided a scale of dimensions, as described earlier (14).

Radioactive Compounds

Radioactive compounds were labeled with carbon-14: adenine, 100 μCi/ml, 50 mCi/mmol (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N.Y.); and serine 100 μCi/ml, 120 mCi/mmol (New England Nuclear, Boston, Mass.). In preliminary experiments we examined several other ^{14}C-labeled compounds to select those with the highest uptake rates when labeling culture volumes of 25 ml. These compounds included arginine, glutamate, glycine, isoleucine, leucine, and valine. Serine and adenine appeared to have much higher uptake rates in YNB medium than any of the other compounds.

Size Selection of Labeled Cells

Exponential phase cultures (25 ml) were exposed for 5 min to labeled adenine or serine at activities of 0.5–1.5 μCi/ml. The cells were then killed and fixed by addition of formaldehyde to a concentration of 3.7%. After 1–2 min the culture was washed six times on a membrane filter (47 mm, 0.25 μm, Schleicher and Schuell, Inc., Keene, N.H.) with 5 ml medium per wash to remove extracellular radioactivity. The cells were then scraped off the filter with a glass rod and resuspended into 0.5 ml of counting solution. After this suspension was subjected to vigorous vortexing for 2 min, 0.3 ml was layered on a sorbitol gradient, 5–30%. The number of cells layered ranged from approximately 2×10^8 to 6×10^8, well within the maximum loading recommended by Mitchison and Vincent (15). With the equipment and methods described earlier (14), cells layered on the gradient were centrifuged at 70 *g* for 1.75–2.5 min. After centrifugation, samples (0.1 or 0.2 ml) of different cell size classes were removed at intervals of $\frac{1}{8}$" along the band. Each was diluted in 1 ml of YNB; 0.5 ml was used for cell counting and sizing, and 0.1-ml volumes were diluted into 5 ml of scintillation fluid to provide replicate samples for counting. The scintillator fluid contained 42 ml of Spectrofluor PPO-POPOP, (Amersham/Searle Corp., Arlington Heights, Ill.) and 100 *g* of napthalene in 1 liter of dioxane. Maximal values of uptake were always less than 3% of the labeled substrate available.

Radioactivities were determined as counts per min per cell as a function of mean cell size. Relative mean cell volumes (RCV) for each sample were obtained by dividing the observed values by the average volume at birth. Average birth volume was determined for each experiment by multiplying the observed value for the mean cell volume in formalin-treated cells from the exponentially growing culture by ln 2, as described earlier (14).

Termination of labeling periods by addition of formalin had no effect on measurements of uptake or incorporation in our experiments. Measured values of uptake did not differ significantly from those when samples were simply washed with growth medium, either for serine and for adenine (experiments not shown). As a further test, corresponding levels of incorporation of these compounds also were measured after exposing the cells to cold 10% trichoroacetic acid (TCA). Values of incorporated radioactivities also remain unchanged in cells exposed to formalin. These results support the stability of the acid-soluble pool to formalin treatment.

DNA Content

Average contents of DNA per cell were determined as described by Hartwell (16). A 25-ml culture was inoculated for overnight growth, [^{14}C]adenine was added to a concentration of 2 μCi/ml, and unlabeled adenine was added to 10 μg/ml. The following day, when the culture was in exponential growth at a cell density between 2 and 3×10^6/ml, 3 ml of 37% formaldehyde was added to the labeled culture. The cells were washed and banded, and samples were removed and diluted as described earlier. DNA content was measured in triplicate 0.2-ml samples, by addition of 0.2 ml of 2 N KOH to each, and hydrolysis at 37°C for 24 h, after which the samples were chilled and 20 μg of carrier calf thymus DNA and 0.2 ml of 50% TCA were added. The samples were then filtered (Whatman GF/C, 2.4 cm, Whatman, Inc., Clifton, N.J.) and rinsed 10 times with 1-ml portions of 5% TCA containing 10 μg/ml unlabeled adenine. The filters were dried under a heat lamp to remove any remaining TCA, and placed in scintillation vials for counting.

RESULTS

When exponential phase cultures were exposed to labeled adenine or serine before the cells were fixed and separated by size, uptake rates for both compounds followed very similar patterns during the cell cycle (Fig. 1). Rates appeared to be constant for about

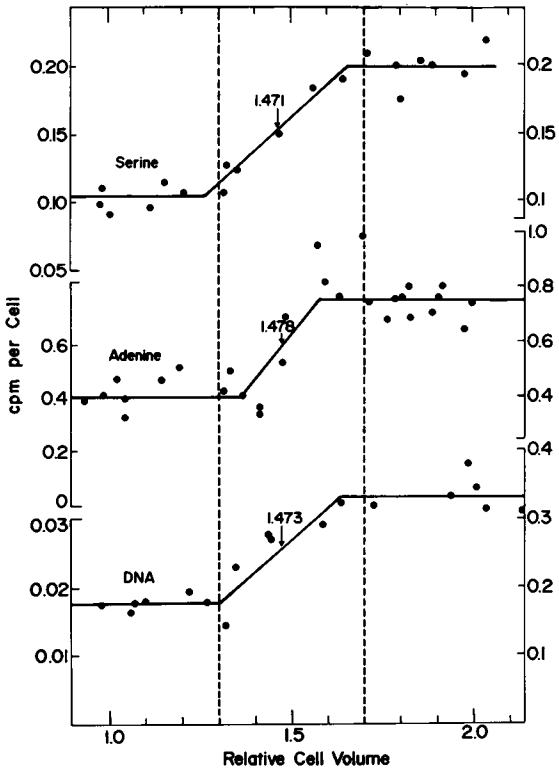

FIGURE 1 Rates of uptake of serine and adenine and content of DNA per cell during the cell cycle in *S. cerevisiae* Y55. Relative cell volume is given in terms of unit mean cell volume at birth calculated on the assumption of linear cell growth, and corresponding values of cell ages are found by subtracting 1.0. The data (three experiments for serine, three for adenine, and four for DNA content) are divided into three periods by the vertical dashed lines. The horizontal lines in the first and third periods are the averages of the data points in those intervals. The lines joining the initial and final plateaus are linear regressions to the data in the middle period. The midpoints of each step are shown by the vertical arrows.

the first third of the cell cycle, and increased during the middle period to final values twice as large during the final third of the cell cycle. Values of DNA per cell increased in the same manner. The relative cell volumes in this figure are those compared with the calculated mean cell volume at birth for cells assumed to grow at constant rates. Cell size distributions obtained by this method of selection have coefficients of variation (C.V.) of about 0.15, about as broad as those obtained with other methods. Narrower distributions would have been more desirable, but were not obtainable.

To show the major features of uptake response during the cell cycle, the observed values were divided into three groups by the vertical dashed lines shown in Fig. 1. Horizontal lines were fitted to the data in the first and third portions of the cycle through the average values of the data points in those intervals. The ratios of the final to initial plateau values (and their standard errors) were 1.91 $\pm$ 0.08, 1.88 $\pm$ 0.08, and

TABLE I

VARIANCE RATIO TESTS COMPARING THE STEPPED FUNCTION INCREASES IN FIG. 1 WITH LINEAR FITS TO THE DATA

		Linear regression		Linear doubling	
	n	Variance ratio	Probability level	Variance ratio	Probability level
Serine	19	3.06	5%	1.99	10%
Adenine	29	2.29	1%	2.30	1%
DNA	18	2.48	5%	2.61	5%

1.87 ± 0.07, respectively, for rates of serine and adenine uptake and for DNA per cell. None of these ratios differs significantly from a doubling. The straight line segments connecting the initial and final plateau values are linear regressions to all of the data points in the middle part of each cycle.

The best values for the times at which uptake rates doubled and for the midpoint of the period of DNA synthesis are the half-values between the initial and final plateaus, shown by the vertical arrows in Fig. 1. The three relative cell volumes for doubling of rates of serine and adenine uptake and for DNA synthesis are 1.471, 1.478, and 1.473, respectively. The average relative cell volume for doubling of serine and adenine uptake rates is 1.474, and does not differ significantly from the corresponding volume for DNA content.

It is important to note that the data in Fig. 1 are not fitted as well by a linear regression through all of the data or by a linear increase throughout one cell cycle (linear doubling) as they are by the stepped increases shown in that figure. These alternatives were compared with the stepped function fit by the variance ratio test; the values are given in Table I. The stepped function was significantly better (5% level) for all cases but one, where the level of significance was 10%.

DISCUSSION

Our results (Fig. 1) indicate that rates of uptake of both serine and of adenine are constant and double abruptly and simultaneously during the cell cycle in *S. cerevisiae* Y55, and that this doubling occurs during DNA synthesis (S phase). The lack of sharpness of this step in our experiments is consistent with the broad size resolution obtained by our procedure (C.V. = 0.15), mentioned earlier. As far as is known, other methods of size selection share the same lack of resolution.

From Fig. 1, the S phase ranges over values of relative mean cell volume (RCV) from about 1.3 to 1.6, corresponding to cell ages of 0.3 and 0.6 generations if growth rates were constant. These values are in agreement with those observed in earlier gradient selection experiments with *S. cerevisiae*. From the data of Sebastian et al. (17; their Figs. 4 and 5), we estimate that DNA synthesis occurred between cell ages of about 0.25 and 0.6 of the cell cycle. Fraser and Carter (18) estimated that the S phase in their experiments included ages from 0.4 to 0.8 generations, although they indicated diffi-

culty in specifying cell ages in the zonal gradient centrifuge. Because their assignment of cell ages allotted the first 20% of the cycle to small cell volumes that included less than 1% of the cell population, we have made a rough correction of their data by deleting the first 20% of their cycle and expanding the remainder. With this correction, the S phase they observed (their Fig. 5) is located between about 0.25 and 0.75 generations. Thus the three determinations appear to be in substantial agreement.

Earlier measurements of uptake of amino acids in synchronized cultures of *S. cerevisiae* by Carter and Halvorson (19) had indicated abrupt doublings of rates of uptake for each. Their results, however, suggested that cell controls were altered since values were extremely variable from experiment to experiment, and even within a given experiment doublings of uptake of leucine and serine occurred at different cell ages during the first and second cycles. Recently, Fraser and Moreno (20) and Mitchison (21) also obtained evidence for alteration of cell characteristics in yeast when these were synchronized by the velocity sedimentation selection technique. Fraser and Moreno performed asynchronous control experiments that revealed that rates of adenine uptake and incorporation into polyadenylated RNA in *S. pombe* were depressed for about 30 min after removal of the cells from the gradient. (The generation time of these cells was 145 min.) Mitchison, using a similar system, found that rates of synthesis of several different enzymes were altered for 40–80 min. In addition, we find that the glucose transport system is extensively perturbed when cells are synchronized by this method (unpublished results). All of these findings indicate that velocity gradient selection of cells perturbs the cell growth cycle and that earlier results based upon this approach require reassessment. The advantage of the method of size selection which we used is that it is free of growth and transport perturbations after labeling.

Our observation that doubling of uptake rates is correlated with the S phase rather than with the end of the cell cycle had not been possible in earlier experiments, because these were performed with cells in which DNA replication occurred so near the end of the cell cycle that it was not possible to distinguish between those alternatives. The midcycle location of the S phase in *S. cerevisiae* in our experiments allows an unambiguous correlation of the doubling of uptake rates with this period, rather than with the end of the cell division cycle as proposed earlier (1, 10). Mitchison's earlier finding that growth rate doubles in single cells of *S. cerevisiae* after an interval after bud emergence (3) also provides support for growth rate doubling at about the same time, since bud emergence correlates with the start of DNA synthesis (22). However, our results do not appear to provide strong support for Sargent's observation with *B. subtilis* that doubling occurs at nuclear segregation (6), and instead suggest that growth rates double earlier in *S. cerevisiae*.

The correlation we have observed between doubling of uptake rates and doubling of the amount of DNA per cell does not not require that uptake rates be directly under genetic control. Hartwell (13) has shown that there are two separate and parallel pathways of events in the cell cycle of *S. cerevisiae*, only one of which concerns DNA syn-

thesis. Controls for uptake may well be located in the alternate events pathway parallel to that for DNA synthesis. In fact, further evidence to support that interpretation has been obtained with synchronous cultures of bacteria: growth rates (and therefore rates of uptake) increased in the absence of DNA synthesis (23, 24).

Whatever the control mechanisms for uptake, however, our observations on uptake rates, taken together with Mitchison's parallel observations on the piecewise constancy of growth rates of single yeast cells, continue to support the hypothesis that this piecewise linear growth is due to the corresponding constancy of rates of uptake of all or almost all of the compounds transported into the cell. This hypothesis predicts constant uptake rates that double in concert with doubling in growth rates, in keeping with our findings for serine and adenine uptake rates.

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