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NOTES

Cell Cycle Phases in the Unequal Mother/Daughter Cell Cycles of *Saccharomyces cerevisiae*

BONITA J. BREWER, EWA CHLEBOWICZ-SLEDZIEWSKA,† AND WALTON L. FANGMAN*
Department of Genetics, SK-50, University of Washington, Seattle, Washington 98195

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During cell division in the yeast *Saccharomyces cerevisiae* mother cells produce buds (daughter cells) which are smaller and have longer cell cycles. We performed experiments to compare the lengths of cell cycle phases in mothers and daughters. As anticipated from earlier indirect observations, the longer cell cycle time of daughter cells is accounted for by a longer G1 interval. The S-phase and the G2-phase are of the same duration in mother and daughter cells. An analysis of five isogenic strains shows that cell cycle phase lengths are independent of cell ploidy and mating type.

At cytokinesis in the yeast *Saccharomyces cerevisiae*, mother cells and daughter cells (the buds) are unequal in size. The smaller daughter cells consequently have a longer cell cycle (4, 5, 8). Most of the increase in cell cycle length in daughter cells occurs in the interval from cell division to emergence of a new bud, and before the step called Start (9; reviewed in reference 11). Since the Start function appears to take place at, or close to, the boundary between the G1-phase and the S-phase, it is likely that the differences in cell cycle lengths for mothers and daughters is entirely accounted for by a difference in the G1-phase (4, 5, 8). This inference, however, has not been tested directly. In addition, it is not known whether the S- and G2-phases are equivalent in mothers and daughters. The results of a direct determination of cell cycle phase durations are presented in this report.

The length of the S-phase in total populations of *S. cerevisiae* has been estimated for only a few strains. The published data indicate that a diploid genome can be replicated faster than a haploid genome (2, 13, 14, 16). The experiments, however, were carried out under different growth conditions and in strains with different genetic backgrounds. We therefore examined the length of cell cycle phases in an isogenic set of haploid and diploid strains under constant growth conditions. In addition, we determined whether mating type influences cell cycle phase durations.

Mother and daughter cells can be distinguished microscopically by a ring of chitin, the bud scar, that forms on the cell surface of the mother at the site of emergence of each bud (1). We coupled the fluorescent staining of bud scars with whole cell autoradiography to determine cycle phase lengths of mother and daughter cells. A culture of the aα diploid A364A D5 (7) growing in glucose minimal medium (13, 18) at 30°C was pulsed with [^{3}H]uracil, collected immediately, and prepared for whole cell autoradiography (13). After exposure and development of the autoradiograms, cells under the emulsion were stained with fluorescent dyes: Calcofluor to reveal bud scars (15) and 4′, 6-diamidino-2-phenylindole to reveal nuclei (17). Mother and daughter cells were scored separately as unbudded, budded with a single nucleus, or budded with two completely separate nuclei (called G1-doublets) (Fig. 1). Budded cells undergoing nuclear division (Fig. 1, arrow) were included in the budded class. The budded cells with two nuclei were interpreted to be cells in which the nuclei, having completed division, had reentered the G1-phase, but the cells either had not completed cytokinesis or had failed to be separated by sonication. Therefore, the completion of nuclear division is taken as the boundary between the G2-phase and the G1-phase. In our analysis, both mother and daughter G1 doublets (ca. 10% of the sample) contribute two cells to the unbudded classes: in each doublet one cell is a mother and one is a daughter. The cycle times of mother and daughter cells were calculated from the proportion of budded mothers and daughters and the culture doubling time (10). The frequencies of labeled and unlabeled cells of the budded and unbudded classes were estimated from the grain-per-cell data (3, 13). These values give the fractions of mother or daughter cells that are in the G1-phase (unbudded, unlabeled), the S-phase (labeled, both unbudded and budded), and the G-2 phase plus nuclear division (unlabeled, budded). The length of each cell cycle phase as a fraction of the total cycle can then be determined (12), and the absolute duration of each phase can be obtained by multiplying by the individual cycle lengths and adjusting for the pulse-labeling time (13).

To establish the reproducibility of this technique for measuring cell cycle phase lengths, duplicate cultures of strain A364A D5 were pulsed, and cells were collected at two intervals during the pulse. For each pulse in the two experiments, at least 1,000 cells were scored, and the data were converted to phase lengths as described above (Table 1). The variations in phase length and cell cycle length measurements (as reflected in the standard deviations) are small within the population of mothers or daughters. The only obvious difference in cycle phases occurs in the contraction of the G1-phase of the mothers relative to the G1-phase of the daughters. No significant differences are seen between mothers and daughters in the S-, G2-, or budded phases.

To investigate the effects of ploidy and mating type on cell

* Corresponding author.
† Present address: Ernst-Boehringer Institute, Bender & Co. Gmbh, Dr. Boehringer Gasse 5-11, Vienna, Austria.

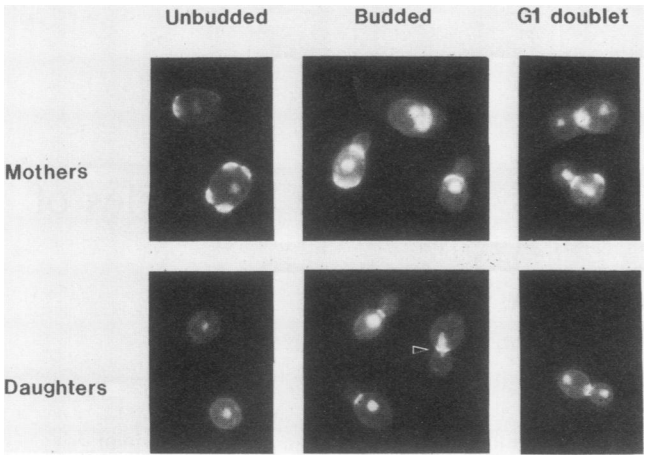

FIG. 1. *S. cerevisiae* cells stained to show bud scars and nuclear morphology. Populations of mothers and daughters were classified into three categories: unbudded cells (unbudded), budded cells which had not completed nuclear division (budded), and cells with two nuclei which had not yet completed cytokinesis or were not separated by sonication (G1 doublet). An arrowhead indicates a cell in early nuclear division. The strain is the aα diploid A364A D5 (7).

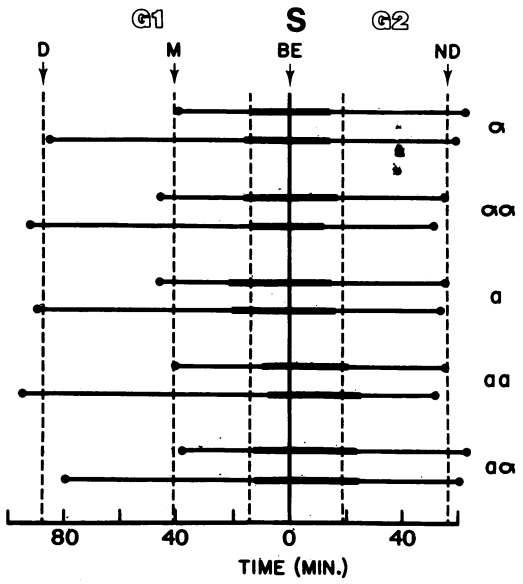

FIG. 2. Cell cycle phase lengths for mother and daughter cells from five isogenic strains. The top line in each set of two represents mother cycles; the bottom line represents daughter cycles. The point of bud emergence (BE) is used to align the cell cycles; nuclear division (ND) is to the right. The time scale places events relative to bud emergence. The vertical dashed lines represent average values for the phase boundaries relative to bud emergence and include the data from all five strains. The isogenic set of strains (**a**, α, **aa**, αα and **a**α) was derived from strain H243-13-2 (*MATa trp1 ura1*), by first selecting a spontaneous α-factor-resistant cell that proved to be an **a**α diploid (L. H. Hartwell, personal communication). The **a**α strain was then either treated with X-rays (6 kilorads) to obtain **aa** and αα strains via mitotic recombination or sporulated to obtain an α haploid via meiosis.

cycle phases, experiments were carried out with five isogenic strains, two haploids (**a** and α) and three diploids (**aa**, αα, and **a**α), derived from strain H243-13-2 which is unrelated to A364A D5. All strains in this set have the same doubling time (120 min at 30°C) in the glucose-minimal medium used. A culture of each strain was pulse-labeled with [^{3}H]uracil, and samples of cells were collected at two or three intervals during the pulse. The cell cycle phase lengths for mothers and daughters in each culture were calculated as described above. As expected, the length of the total cell cycle is shorter for mothers than for daughters (Fig. 2); however, the cycle times for mothers (average, 99 ± 2.9 min) and daughters (average, 142 ± 2.0 min) are not significantly different among the five strains. Between mothers and daughters there is little variation in the budded interval, the S-phase, or the G2-phase. The only significant difference in cell cycle phases is the length of the G1 phase between mothers and daughters. In the schematic representation of the data (Fig. 2), cell cycles are aligned at the emergence of the bud; however, the same general features are apparent when nuclear division is used to align them. Although minor differences are observed among strains, the striking features

TABLE 1. Mother and daughter cell cycle phases in strain A364A D5

Expt	Pulse length (min)	Cycle time (min)	Length of phase (min)			
			G1	S	G2	BI
Mothers						
I	1	78	20	29	29	56
	3	77	18	32	27	53
II	1	81	23	29	29	55
	2	81	21	32	28	49
	Avg ± SD	80 ± 2.1	21 ± 2.1	31 ± 1.7	28 ± 1.0	53 ± 3.1
Daughters						
I	1	89	31	24	34	55
	3	90	34	27	29	52
II	1	97	41	27	29	54
	2	98	43	27	28	48
	Avg ± SD	93 ± 4.7	37 ± 5.7	26 ± 1.5	30 ± 2.7	52 ± 3.1

[a] The culture doubling time was 84 min in experiment I and 90 min in experiment II. BI, Budded interval of the cell cycle.

are the appearance of the bud approximately midway through the S-phase, the invariant lengths of S- and G2-phases, and the threefold shortening of the G1-phase in mothers.

The experiments reported here confirm directly what has been previously inferred from indirect experiments (4, 5, 8). The longer cell cycle of *S. cerevisiae* daughter cells compared with that of *S. cerevisiae* mother cells is accounted for by a longer G1-phase. In addition, the experiments show that S- and G2-phases are of the same duration in mothers and daughters. Similarities among the five isogenic strains in the length of mother cycles, daughter cycles, and budded intervals (Fig. 2) indicate that neither ploidy nor mating type influence significantly the basic mechanisms of cell division. The observation that the S-phase begins at the same cell age in isogenic diploids and haploids, which differ twofold in size, is consistent with a model of cell division control, in which reaching a critical size, monitored as the ratio of some cell component to DNA content, is the controlling event (9, 11).

The appearance of the bud has frequently been assumed to signal the initiation of DNA synthesis. However, there have been few observations directly correlating bud emergence and the boundary between the G1-phase and the S-phase (13). In the aα diploid A364A D5, the bud does appear at a point in both mother and daughter cycles that is close to the entry of cells into the S-phase (Table 1). However, in none of the five isogenic strains derived from strain H243-13-2 does the emergence of the bud coincide with the beginning of the S-phase, but it instead occurs on the average 40% of the way through the S-phase (Fig. 2). Genetic variation in the temporal relationship between the emergence of the bud and the replication phase of the cell cycle is anticipated from the observation that these two processes can be uncoupled in temperature-sensitive mutants (4, 9, 11). It also is clear from earlier work (13, 14, 16) and from the results presented here that considerable strain-to-strain variation exists in the absolute duration of different cell cycle phases, as well as the percent of the cycle devoted to each phase.

We have used synchronized cultures (6) to determine the time of replication of individual sequences in the *S. cerevisiae* genome during the S-phase. The results presented here, that mother and daughter cells have an S-phase of the same duration, give us at least minimal assurance that the replication mechanics of mother and daughter S-phases in these synchronized populations are likely to be the same.

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