Size Control in a Small-size Mutant of *Saccharomyces cerevisiae*

By P. G. LORD*† AND A. E. WHEALS

Microbiology Group, School of Biological Sciences, University of Bath, Bath, UK

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The kinetics of cell proliferation of a population of a small-size mutant of *Saccharomyces cerevisiae* were compared with those of a wild-type cell population, using time-lapse cinephotomicrography. The mean size of small-size mutant cells was approximately half that of wild-type cells at corresponding points in the cell cycle. The cycle times of small-size mutant cells were much more variable, especially for daughter cells, than those of wild-type cells. The difference in the variability of cycle times of the two strains was mainly due to the different degree of variability of their respective unbudded periods. Like wild-type cells, daughter cells of the small-size mutant were smaller and had a longer cycle time than parent cells. The small-size mutant retains a single size control over the cell cycle.

INTRODUCTION

Control of cell proliferation in the yeast *Saccharomyces cerevisiae* is exerted at a single point in the cell cycle which has been defined physiologically and genetically and is called 'start' (Hartwell *et al.*, 1974; Pringle & Hartwell, 1981). Haploid cells will arrest at start if subject to nutrient starvation or in response to mating pheromone. In the absence of these constraints the major rate-determining step for the traverse of start is achievement of a critical cell size (Hartwell & Unger, 1977; Carter & Jagadish, 1978; Tyson *et al.*, 1979) or, more precisely, there is a higher probability that a cell will enter a cycle as it grows in size (Lord & Wheals, 1981; Wheals, 1982).

Schizosaccharomyces pombe has an analogous size control over mitosis (Nurse, 1975; Nurse & Thuriaux, 1977; Nurse & Bissett, 1981), and *wee* mutants which have a defective size control mechanism have been isolated (Nurse, 1975; Thuriaux *et al.*, 1978; Fantes, 1981). These mutants nevertheless still retained a size control mechanism for homeostatic cell size regulation because the *wee* mutation had revealed a second, cryptic size control over DNA synthesis.

The discovery of size control (*whi*) mutants in *S. cerevisiae* (Sudbery *et al.*, 1980; Carter & Sudbery, 1980) provided the opportunity to determine whether there was a second size control or whether cells were proliferating with a defective or absent size control mechanism. Using time-lapse cinephotomicrography we have compared the kinetics of cell proliferation of a *whi1* mutant with those of its *whi1*⁺ parent.

METHODS

Strains. Two haploid strains of *S. cerevisiae* were used: S67.3a (a, *lys2*, *whi1*) and its immediate parent SA (a, *lys2*), obtained from P. E. Sudbery, University of Sheffield, UK.

Media. The strains were maintained on YEP glucose agar (20 g glucose, 20 g bacteriological peptone, 10 g yeast extract, 20 g agar and 1 litre distilled water) supplemented with lysine (0.05 mg l⁻¹). For time-lapse cinephotomicrography the cells were grown on YEP glucose/PVP agar supplemented with lysine [3 g glucose, 3 g bacteriological peptone, 1.5 g yeast extract, 7 g purified agar, 28 g polyvinylpyrrolidone (PVP-40, Sigma), 5 g lysine and 100 ml distilled water].

Time-lapse cinephotomicrography. Each strain was filmed at 25 °C. Full details of the equipment used, of the method of filming and of the analysis of the films have been published previously (Lord & Wheals, 1981).

† Present address: Biochemistry Department, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK.

Table 1. *Mean duration of cell cycle periods of parent and daughter cells of strains SA and S67.3a*

All values are in min with SD in parentheses. 36 parent and 36 daughter cycles were monitored for strain SA cells; 32 parent and 32 daughter cycles were monitored for strain S67.3a cells.

Period	Strain SA		Strain S67.3a	
	Parent cells	Daughter cells	Parent cells	Daughter cells
Total cycle	92.7 (10.3)	136.6 (37.8)	133.1 (24.4)	198.1 (87.3)
Unbudded period	8.2 (6.2)	52.9 (38.2)	24.5 (18.4)	89.2 (87.9)
Budded period	84.5 (10.0)	83.7 (7.6)	108.5 (16.2)	108.8 (17.4)
Bud emergence to nuclear migration	46.1 (9.3)	45.4 (8.3)	68.4 (15.6)	70.4 (15.8)

Table 2. *Mean volumes of daughter cells of strains SA and S67.3a at three stages of the cell cycle*

Results are given in μm^3 with SD in parentheses.

Strain	Birth	Bud emergence	Cell separation
SA	37.2 (6.9)	48.3 (7.5)	53.0 (7.6)
S67.3a	16.3 (5.3)	20.2 (5.4)	21.7 (5.0)

RESULTS

Time-lapse cine films were made of strain S67.3a (*whi1*) and strain SA (*whi1*⁺) cells growing on YEP glucose/PVP agar supplemented with lysine at 25 °C. For each strain the mean cycle times of first and second generation daughter cells were equivalent, as were the mean cycle times of parent cells in their first and in their second cycles after time 0. The mean sizes of first and second generation daughter cells were equal at birth, at bud emergence and at cell separation. Cell number and total cellular volume of each strain increased exponentially throughout the experiment from time 0. Therefore, by these criteria the data are appropriate for a kinetic analysis. The population doubling time (τ) and the population volume doubling time (τ_v) of strain S67.3a cells were 161.8 and 169.5 min, respectively, and 119.1 and 136.5 min for strain SA cells. The differences between τ and τ_v are of comparable magnitude to those of previous experiments (Lord & Wheals, 1981, 1983). The higher value for τ_v probably reflects inaccuracy in measurement as the population becomes crowded.

The mean duration of the cell cycle and constituent periods are given in Table 1 for parent and daughter cells of each strain. Both strains showed patterns typical of *S. cerevisiae* cells growing at different growth rates (Lord & Wheals, 1981). The major differences between the strains were (i) an elongated and more variable budded period in strain S67.3a cells (entirely due to the period between bud emergence and nuclear migration) and (ii) an elongated and more variable unbudded period (particularly for daughter cells) in strain S67.3a cells, the coefficient of variation rising to 99% for strain S67.3a daughter cells.

The distributions of the parent and daughter cycle times of both strains are presented as α plots (Smith & Martin, 1973) in Fig. 1. For both strains the α plot of parent cycle times is 'steeper' than that of daughter cycle times. As was the case for strain A364A cells (Lord & Wheals, 1981), the shape of the plot of daughter cycle times was determined mainly by the distribution of the lengths of the unbudded period (Fig. 2). It should be noted that in interpreting these figures no conclusions can be drawn from the shape of the 'tails' of the plots since they represent only 10% of the data (in these examples, data from three cells).

Fig. 1. α plots of parent and daughter cycle times of strains SA and S67.3a. The percentage of cells with cycle times of duration (t_c) greater than or equal to t (on a logarithmic scale) versus t . $\circ$, $\bullet$, Strain SA cells; $\square$, $\blacksquare$, strain S67.3a cells; open symbols, parent cells; filled symbols, daughter cells.

Fig. 2. α plots of the unbudded periods of parent and daughter cells of strains SA and S67.3a. The percentage of cells with unbudded periods of duration (t_{ub}) greater than or equal to t (on a logarithmic scale) versus t . $\circ$, $\bullet$, Strain SA cells; $\square$, $\blacksquare$, strain S67.3a cells; open symbols, parent cells; filled symbols, daughter cells.

Fig. 3 shows the distributions of daughter cell sizes at birth and at bud emergence. The distribution of birth sizes overlaps with the distribution of bud emergence sizes for both strains although more so for strain S67.3a. The mean values of the size of daughter cells at three cell cycle stages are given in Table 2. The mean size of strain S67.3a cells was less than half the size of strain SA cells at the corresponding stages.

Plots of size at cell separation (birth) against the duration of the subsequent unbudded period reveal the relative importance of size in determining the length of the unbudded period, the latter being taken as an approximate measure of the pre-start period (Lord & Wheals, 1981). The data for both strains, plotted in this way, are shown in Fig. 4. The graph for strain SA cells is similar to those obtained for strain A364A cells (Lord & Wheals, 1981), except that some of the points for the daughter cells are clustered directly beneath the points for the parent cells.

Fig. 3

Fig. 3. Distribution of the size of daughter cells of strains SA (a) and S67.3a (b) at birth (solid line) and at bud emergence (broken line).

Fig. 4

Fig. 4. Cell size at cell separation versus the duration of the subsequent unbudded period. (a) Strain SA cells; (b) strain S67.3a cells; open symbols parent cells; filled symbols, daughter cells. The correlation coefficients of daughter cell size at cell separation versus the duration of the unbudded period are (a) -0.54 and (b) -0.70 .

The graph for strain S67.3a cells is different in several ways: (i) there is higher correlation between the birth size of daughter cells and the duration of the unbudded period ($r = -0.70$ compared with $r = -0.54$ for strain SA cells); (ii) size does not appear to be important in determining the length of the unbudded period for parent cells, although some of the parent cells whose birth size was less than $20 \mu\text{m}^3$ did have longer unbudded periods than the other parent cells; (iii) in comparison to strain SA parent cells, the larger of the strain S67.3a parent cells have a broader distribution of times spent in the unbudded period; and (iv) whereas for strain SA cells the data points for parent and daughter cells form discrete clusters, the points for strain S67.3a cells interdigitate.

DISCUSSION

The comparison of these strains is rendered difficult because of the difference in the growth rates under equivalent environmental conditions. It is known that the size at bud emergence (and by inference the size at start) decreases as the growth rate decreases (Johnston *et al.*, 1979; Tyson *et al.*, 1979). However, the magnitude of the difference in size at bud emergence between these strains was too great to be simply due to the modest difference in the growth rates.

Is there evidence for a size control mechanism to be still operating, as suggested by Carter (1981)? If there is, it must reside in the unbudded period (as in wild-type cells) because that is where the bulk of cycle time variability exists. Two pieces of evidence point to the existence of a size control. (i) There is still the difference in cycle times of parent and daughter cells, which is caused in part by a size control operating at start (Hartwell & Unger, 1977; Lord & Wheals, 1981) and is largely abolished in cells where cell size is not a rate-determining step in cell proliferation (Lord & Wheals, 1983). This is confirmed by the asymmetry in size at division

between parent and daughter cells. (ii) There is a pronounced correlation between birth size of daughter cells and the duration of their subsequent unbudded periods (Fig. 4), consistent with the hypothesis that smaller cells have to grow more, on average, than larger cells before initiating a cycle. At faster growth rates wild-type cells produce large daughter cells which are able to bud after a short duration (Fig. 2). This leads to there being a narrow scatter of the lengths of the unbudded periods, compared to *whi1* cells, and thus the correlation coefficient is less. A plot of birth size versus bud emergence size (data not shown) gives a weak correlation ($r = 0.21$) since these should be independent of one another if there is size control.

There are some differences between *whi1* and *whi1*⁺ cells. Most noticeably, *whi1* cells are less than half the volume of *whi1*⁺ cells at comparable stages in the cell cycle (Table 2). Furthermore, there is more variability in the size and time at which the cycle is initiated in *whi1* cells (Table 1). The kinetics of entry into the budded phase of wild-type parent and daughter cells are $p = 0.9999 \text{ h}^{-1}$ and 0.9400 h^{-1} , respectively, where p is the probability per unit time of the appearance of a bud on an unbudded cell. For *whi1* cells the slopes of the curves are not convincingly exponential but the slopes for parent and daughter cells give values of $p = 0.81 \text{ h}^{-1}$ and $p = 0.48 \text{ h}^{-1}$, respectively. For both strains, parent and daughter cells have markedly different kinetics.

Two models have been proposed to account for the kinetics of proliferation of yeast cells (Lord & Wheals, 1981; Wheals, 1982) and both can, in principle, accommodate the altered size and variability properties of *whi1* cells. The tandem model, which presupposes separate size control and probability components, would require an alteration in the parameters of both aspects of control to be caused by a lesion in a single genetic locus (Carter & Sudbery, 1980). The alternative model, the sloppy size control model, is a one-component model in which the interaction of a protein with a size-dependent effector is the rate-determining step for proliferation (Wheals, 1982). It is feasible that a single molecular change would alter the affinity constant of the protein for the effector and so would alter the measured parameters. In the absence of any molecular information on this point a numerical value cannot be given to the change required.

Although *whi1* cells initiate their cycle at half the volume of *whi1*⁺ cells, size control is still retained, presumably at start. Sudbery *et al.* (1980) found that the mean size (of budded parent cells) of *whi1* haploids, *whi1/whi1* diploids, *whi1*⁺ haploids, *whi1/whi1*⁺ diploids and *whi1*⁺/*whi1*⁺ diploids are roughly in the ratio 1:2:2:3:4. This observation led Sudbery *et al.* (1980) to suggest that the small size of *whi1* cells is due to a gene dosage effect. They hypothesized that there are two genes (*whi1* mutants defining one of them) whose products act in parallel, the cell size at bud emergence (and by inference, at start) being proportional to the combined number of the two genes. However, the two genes need not necessarily be different. An alternative hypothesis to explain the gene dosage effect is that the *whi1*⁺ gene is present in two copies per haploid complement (duplicate gene hypothesis). In this hypothesis a *whi1* mutant would contain one defective and one functional copy of the *whi1*⁺ gene.

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