SMALL-SIZED MUTANTS OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

The isolation of mutants of *Saccharomyces cerevisiae* that divide at approximately half the size of the wild type is described. Three mutants have been isolated in which the small size at bud initiation is due to a mutation in a single nuclear gene.

THE processes by which *Saccharomyces cerevisiae* coordinates growth and cell division has been the subject of considerable interest (HARTWELL and UNGER 1977; JAGADISH, LORINCZ and CARTER 1977; JOHNSON, PRINGLE and HARTWELL 1977; CARTER and JAGADISH 1978). An important conclusion from these studies is that mother cells must attain a critical size before bud formation and consequent commitment to division. The control of cell size and division are thus aspects of a common process.

New insights into this process might be obtained by the use of mutants that divide at an abnormal cell size. Mutants that divide at a smaller size than normal have been obtained in the fission yeast, *Schizosaccharomyces pombe*, using a technique based on velocity separation through a sucrose gradient, which separates cells according to size (THURIAUX, NURSE and CARTER 1978). Studies with these mutants have shown that in the fission yeast there is a size control acting at nuclear division (NURSE 1975; FANTES and NURSE 1977) and another size control that is cryptic in fast-growing cells at the initiation of DNA synthesis (NURSE and THURIAUX 1977).

We report here on the isolation of mutant strains of *S. cerevisiae* that initiate bud formation at a smaller-than-normal parent cell size. Initially, we attempted to use the procedure involving sedimentation velocity in a sucrose gradient developed for *S. pombe*. Reconstruction experiments using diploids and haploids indicated that this should provide a 15,000-fold enrichment for small cells. We failed, however, to recover any small mutants when this method was used to enrich for mutants, using a haploid as the parent strain for mutagenesis.

Subsequently, we devised a novel method based on the use of the α-factor mating hormone to isolate small mutant haploid strains. These strains initiate bud formation when both the volume and dry mass of the cell are half the value of

the parent strain from which they were derived. The size reduction in three of the mutants is caused by a single nuclear mutation, as shown by its 2:2 segregation in the progeny of a cross to a normal-sized haploid strain.

MATERIALS AND METHODS

Strains and media: Haploid strains X2180–1A (**a** *SUC2 mal1 gal2 CUP1*) S30 (*αmet3 leu2-1*) and AN33 (*αthr1 arg1*) were kindly provided by the Berkeley Yeast Stock Center. Strains were routinely cultured at 25° in shake flasks containing YEPD liquid or on YEPD agar plates. Tests for gal⁻ phenotype was performed on plates containing 0.1% galactose in synthetic complete medium. Mal⁻ was scored using brom-cresol purple medium (SHERMAN, FINK and LAWRENCE 1971).

Genetic analysis: Mating by prototrophic selection, sporulation and microdissection of tetrads were all carried out according to standard methods described by SHERMAN, FINK and LAWRENCE (1971).

Cell volume measurements: Cells were grown to stationary phase in shake flasks containing YEPD. They were diluted into fresh YEPD, and after 7 hr at 25° photomicrographs were made at 400× magnification. Negatives were projected onto a screen, using a slide projector, and the major and minor axes of the parent cell portion of cells with buds were recorded for 30 cells. The volume of cells was then calculated (using a stage graticule as a standard), assuming yeast to be a prolate spheroid.

Dry mass measurements: Stationary-phase cultures were diluted in fresh medium as described for cell volume measurements. Cell samples were taken and mixed with a 17.5% gelatin solution at 32°. A drop of this liquid mixture was placed on a slide, covered with a coverslip and placed at room temperature for a few minutes to allow the gelatin to solidify, thereby immobilizing the cells. The dry mass of cells that had just initiated bud formation was measured with a Vickers M86 scanning interferometer (GOLDSTEIN 1977).

Velocity separation using zonal centrifugation: Cells growing exponentially were harvested and homogenized in an M.S.E. homogenizer (M.S.E., CRAWLEY, England) for 90 sec to separate clumped cells. The cell suspension was layered onto a 15 to 40% sucrose gradient made up in growth media in a Type A zonal motor (M.S.E., CRAWLEY, England). Cells were then separated according to size, as previously described (SEBASTIAN, CARTER and HALVORSON 1971; MITCHISON and CARTER 1975).

α-factor: α-factor was prepared and assayed according to the method of BÜCKING-THROM *et al.* (1973).

Mutagenesis: A 250 ml culture was allowed to grow into stationary phase in YEPD medium. The cells were harvested by centrifugation and resuspended in 250 ml of 0.1 M of phosphate buffer, pH 8. 2.5 ml of EMS (Sigma) was added and the suspension vigorously shaken to ensure dispersal of the EMS. The cells were left standing for 60 min. At the end of this period they were reharvested by centrifugation and washed twice in water before being resuspended in 1 l of YEPD medium. They were then allowed to grow to stationary phase, both to allow expression of any mutations and for the reasons described in RESULTS.

RESULTS

Isolation of small haploid mutants: Haploid cells of α mating type produce a substance, α-factor, that specifically inhibits the division of **a** cells (LEVI 1956; DUNTZE, MCKAY and MANNEY 1970; THROM and DUNTZE 1970). α-factor arrests **a** cells at a specific point in the cycle prior to the initiation of DNA synthesis and bud formation (BUCKING-THROM *et al.* 1973). Cells arrested by α-factor continue to grow in size, producing large unbudded, misshapen cells known as "schmoos." We reasoned that α-factor arrest of **a** cells could be used to isolate small mutants as follows: In a synchronous culture of normal-sized

cells containing mutants that produce a bud at a smaller size than normal, it should be possible to add α-factor to the culture at a time before the α-factor-sensitive step for normal cells, but at a time when mutant cells will have initiated bud formation and will consequently be insensitive to α-factor for one cell cycle.

The method consisted of mutagenizing a culture of X2180–1A and growing it to stationary phase at 25° in YEPD liquid media. The culture was then separated according to size and a homogeneous fraction of small cells was inoculated into fresh YEPD media at 37°. The median cell volume of these cells was 16 μm^3. The starting inoculum contained no budded cells and all were classified as age 0 cells on the basis that they lacked bud scars. Their subsequent growth was monitored using a Coulter Channelizer. When the median cell volume reached 25 μm^3, α-factor was added to a final concentration of 8 units/ml. Since age 0 wild-type cells are sensitive to α-factor until they reach 38 μm^3 (LORINCZ, personal communication), division of these cells was arrested, but they continued to grow in size and formed large schmoos. Any mutant cells that at the time of α-factor addition had already initiated bud formation would escape the action of the α-factor and would be arrested only after division of the mutant cells. These would start to form schmoos during the second cycle. They would be much smaller than the schmoos formed by wild-type cells from which they can, therefore, be separated by zonal centrifugation.

After six hr at 37°, the cells were separated according to size and cells from the earliest fractions recovered from the center of the rotor were plated out and screened as before. A single mutant colony was isolated from approximately 1,000 micro-colonies examined on the basis that cells in the micro-colony were abnormally small. The isolation procedure was repeated twice and small mutants were isolated at a frequency of approximately 0.1% of the colonies screened.

Characterization of the small mutants: Although several small mutants were isolated each time we used the isolation procedure described above, we analyzed only one mutant from each mutagenized culture to avoid clonally derived mutants. Three mutants, *whi-1-A1*, *whi-1-C7* and *whi-1-D3*, derived from these different mutagenized cultures, were examined in detail. Each expressed the fermentation markers of the parent strain, X2180–1A, and mates with wild-type-sized cells of the opposite mating type. They are, therefore, unlikely to be contaminants. Parent cell volume of mutant cells at bud initiation (from photographs) and dry mass measurements of mutant cells with very small buds (from scanning interferometry) were compared with that of the parent X2180–1A Table 1).

The small mutants were mated to haploids of the opposite mating type that divide at the wild-type size. *whi-1-A1* was mated to S30, and both *whi-1-C7* and *whi-1-D3* were mated to AN33. The resulting diploids were placed on sporulation media and, although sporulation efficiency was low (*ca.* 15%), most tetrads gave four spores that germinated to form healthy colonies. Cell volume at bud initiation of segregants from 12 tetrads from a cross of *whi-1-1A* × S30 is shown in Table 2. The size difference between the *whi-1-1A* phenotype and the wild

TABLE 1

Cell volume (μm^3) at bud initiation of parental and mutants strains

Strain	Culture temperature (°C)	Cell volume (μm^3)	Cell mass (arbitrary units)
X2180-1A	25	47 ± 2	11.5 ± 0.4
X2180-1A	37	48 ± 2	—
whi-1-A1	25	23 ± 1	5.4 ± 0.5
whi-1-A1	37	22 ± 2	—
whi-1-C7	25	22 ± 3	—
whi-1-C7	37	24 ± 2	—
whi-1-D3	25	19 ± 1	—
whi-1-D3	37	22 ± 2	—
X2180-1A × X2180-1B	25	78 ± 3	19.5 ± 1.2
whi-1-A1 × AN33	25	66 ± 3	—
whi-1-C7 × AN33	25	62 ± 3	—
whi-1-D3 × AN33	25	65 ± 4	—

type is sufficiently marked for the trait to be reliably scored by microscopic examination of liquid cultures. An additional 30 tetrads were examined by this method, and the small phenotype was found to segregate 2:2 in all of them. Both *whi-1-C7* and *whi-1-D3*, when crossed to wild-type cells (AN33), also segregated the small phenotype 2:2 when the meiotic products of 20 diploids of each were examined. We conclude, therefore, that the small phenotype of each of the three mutants is a result of a single nuclear gene mutation.

Each mutant was crossed to a wild-type strain, and the parent cell volume at budding of the resulting diploids was determined. In each case, the size of the heterozygotes was intermediate between that of wild-type haploids and diploids (Table 1). Therefore, each mutant allele is co-dominant with the wild-type allele. This makes complementation testing difficult.

TABLE 2

Cell volume (μm^3) of segregants from whi-1 × *S30 diploids*

Tetrad no.	Cell volume (μm^3) of spore clones			
	A	B	C	D
1	26.0	19.2	40.4	48.6
2	43.3	41.7	24.8	30.9
3	39.7	25.9	23.7	45.9
4	24.8	46.5	35.2	24.5
5	46.1	34.5	29.3	24.6
6	46.2	22.5	22.2	47.0
7	49.6	22.8	22.2	45.7
8	26.6	49.9	36.6	26.2
9	43.2	41.2	26.8	29.9
10	54.3	22.2	20.8	49.2
11	47.3	25.4	23.2	35.9
12	26.0	43.9	48.7	22.8

To determine how many genes were defined by the three mutants, we crossed each mutant to AN33 and S30 and isolated small progeny with combinations of mating type and auxotrophic markers that permitted mating of *whi-1-A1* × *whi-1-C7*, *whi-1-A1* × *whi-1-D3* and *whi-1-C7* × *whi-1-D3* and selection of diploids by prototrophic selection. These double-mutant diploids were sporulated and tetrads dissected. Spore clones from each tetrad were grown to exponential phase in liquid YEPD, and cell size at bud initiation was determined from photomicrographs. Thirty cells were measured for each determination. Spore clones of 20 tetrads from each diploid combination were analyzed, but wild-type recombinant progeny were not observed. It is likely, then, that the three mutants have mutations in the same gene, although the data are not sufficient to rule out the possibility of separate, but closely linked, genes.

DISCUSSION

Small mutants were successfully isolated with the α-factor technique, which exaggerates the difference in size of small mutants and wild-type haploids. During incubation with α-factor, the latter reach a size larger than that of wild-type cells at the end of their cycle.

The mutant strains were half the size of the wild-type parent when cell volume at bud initiation was determined from photographic measurements of cells in liquid culture. The *whi-1-A1* strain was shown to be smaller than the wild-type parent, both on the basis of volume, measured from photographs, and dry mass, measured with a scanning interferometer. The use of photographs to measure volume is a common procedure, but may be subject to two errors. First, it is necessary to make assumptions concerning cell shape that may not be strictly accurate. Second, cells may contain vacuoles that do not form part of the cytoplasmic mass, but that will nevertheless affect cell volume. The dry mass measurements are free from both of these sources of error. Our studies indicate that the ratio of wild-type cell size to mutant size is very similar whether cell volume or dry mass is used as a measure of size. Other experiments (SUDBERY, unpublished) show that the size ratio of the haploid X2180–1A to the diploid C276 is also identical whether volume or dry mass is used as the measure. It may be concluded that volume measurements as a parameter of cell size are not seriously affected by the possible errors described above.

We should emphasize the difference between small mutants and "petites." Small mutants are cells that initiate a bud at a smaller size than normal. Colony size may be the same as observed for wild-type cells. Small mutants are not necessarily respiratory deficient; indeed, the mutants described here are respiratory competent. Petites are respiratory-deficient cells. Colony size is smaller than that observed in wild type on YEPD medium, but the individual cells in the colony produce a bud when the parent cell reaches the wild-type size. It is possible, however, that our enrichment technique will result in the isolation of petites, as well as small mutants, since size at bud initiation is dependent on growth rate (JOHNSTON *et al.* 1979) and petites grow more slowly than wild-type cells on YEPD medium.

The procedure that we have described should be useful in the isolation of conditional temperature-sensitive (ts) mutants, and thus allow a determination of when during the cell cycle genes involved in the coordination of growth and division exert their effect. Even in the absence of ts mutants, genetic analysis of a collection of small mutants should permit identification of the number of genes involved in this coordination.

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