

Isolation of Monosomics in Yeast

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Tetraploid cultures of *Saccharomyces cerevisiae* triplex (A/A/A/a) at several loci were sporulated. All the diploid spores are expected to be homozygous (A/A) or heterozygous (A/a) and, hence, to have the dominant phenotype. Cells lacking one of the chromosomes (monosomics) may show the recessive phenotype (a/-). Therefore, spores that grew on complete medium but failed to grow on the relevant synthetic single-omission media were presumed to be monosomic. These isolates were further characterized by sporulation, and several stable monosomics were established.

Monosomics are cells diploid for all chromosomes but one, which is present in the haploid condition. They have been identified as occasional products of meiosis in tetraploid strains of yeast (5). The four ascospores resulting from a single meiosis in a tetraploid are expected to be diploid, but a nondisjunctional event may result in the production of an ascus that contains one trisomic, one monosomic, and two diploid spores (5). In a tetraploid of the genotype A/A/A/a at a given locus, a normal meiosis would result in two spores of the genotype A/A and two of the genotype A/a. All spores would have the phenotype conferred by the dominant allele. The appearance of spores with the recessive (a) phenotype can occur by mutation, gene conversion, or nondisjunction. Hence, the recovery of phenotypically recessive spores from triplex (A/A/A/a) tetraploids constitutes a procedure for the isolation of specific monosomics. The monosomic condition may be confirmed by sporulation or further crosses.

Extensive genetic mapping has been done on *Saccharomyces cerevisiae* using tetrad analysis (3). This approach, however, is laborious when the genes to be located are not centromere-linked. Part of the motivation for designing a method of obtaining monosomics was the hope that they might be useful for mapping. When a monosomic is sporulated, half the resulting spores are nullisomic, i.e., they are haploid cells that lack the chromosome which was monosomic in the parent. The other spores are normal haploids. If the nullisomic spores can be induced to mate with haploids, then the resulting monosomics will have the same phenotype as the haploids for any markers located on the monosomic chromosome. To map a recessive gene, a haploid carrying that recessive gene would be mated with the spores of

a sporulated monosomic carrying the dominant allele, and the resultant hybrids would be selected. Only if the unlocated marker is on the monosomic chromosome would any of the hybrids have the recessive phenotype. In that case, half the zygote clones, or all the hybrids resulting from the mating of the haploid with nullisomic (N-1) spores, would have the recessive phenotype.

Monosomics are quite interesting for other reasons. Since the missing chromosome will segregate as a centromere-linked lethal, centromere-linked genes present in heterozygous condition (B/b) in an a/α monosomic will segregate mostly 2B or 2b in the two viable spores. Consequently, a monosomic for any chromosome may be used to determine in one cross whether any segregating gene is centromere-linked. Entire chromosomes may be mapped by the isolation of mutants in monosomic strains. Most mutations are recessive and if isolated in the monosomic must be on the monosomic chromosome. Recessive genes affecting meiotic recombination are best isolated in monosomics, where their effects can be assayed directly by sporulation.

MATERIALS AND METHODS

Media and techniques. Media and techniques are as described in Hawthorne and Mortimer (2).

Yeast strains. Two tetraploid strains of *S. cerevisiae* were used. The first (XB105) was constructed from two diploids, X2939 and Y02587-10, by a forced mating (4). X2939 has the following genotype:

$\frac{a}{\alpha} \frac{ade1-1}{+} \frac{gal1}{+} \frac{trp1-1}{+} \frac{ura3}{ura3} \frac{his2-1}{+} \frac{leu1-1}{+} \frac{arg4}{+}$

$\frac{met14}{+} \frac{asp5}{+} \frac{gal2}{+} \frac{lys7}{+}$

Y02587 has the genotype:

$$\frac{a \ trp1-1 \ ura1}{a \ trp1-1 \ ura1}$$

The resulting tetraploid was triplex (A/A/A/a) at the loci *ade1* (chromosome I), *gal1* (chromosome II), *his2* (chromosome VI), *leu1* (chromosome VII), *arg4* (chromosome VIII), *met14* (chromosome XI) *asp5* and *gal2* (chromosome XII), and *lys7* (chromosome XIII). The mating mixture was incubated for 2.5 hr on glucose nutrient agar (GNA) and then streaked onto complete-uracil for the selective growth of tetraploid zygotes. One of the tetraploids from this cross was sporulated and dissected.

The second tetraploid culture used (X1221a) has the following genotype:

$$\frac{a \ ade1 \ gal1 \ trp1 \ ura3 \ thr3 \ his2 \ leu1 \ ade6 \ arg4 \ thr1 \ Cu}{\alpha \ + \ + \ + \ + \ + \ his2 \ + \ + \ + \ + \ + \ cu}$$

X1221a is triplex at the loci *ade1* (chromosome I), *gal1* (chromosome II), *trp1* (chromosome IV), *ura3* and *thr3* (chromosome V), *leu1* and *ade6* (chromosome VII), *arg4* and *thr1* (chromosome VIII), *gal2* (chromosome XII), *lys7* (chromosome XIII), and *ade2* and *ade9* (fragment 1). This tetraploid was sporulated, and the asci were treated with snail enzyme. The asci were disrupted by sonic treatment, and the resulting mixture of diploid spores and tetraploid parent cells were plated out to a density of about 100 per petri plate on yeast extract, peptone, and dextrose (YEPD). Colonies that failed to grow after replica-plating on minimal medium plus methionine and histidine or on yeast extract plus galactose medium were presumed to be monosomics. They were checked for their ability to use galactose as a carbon source and for growth on a series of synthetic single-omission media lacking uracil, leucine, arginine, threonine, tryptophan, or lysine.

RESULTS

Sixteen asci from a sporulated culture of the tetraploid XB105 were dissected, and their spores were scored on omission media (Table 1). The results are consistent with the genotype above, and one of the spores (XB105-16C) appears to be monosomic for chromosome VI, since it has the recessive phenotype conferred by *his2-1*, which is located on that chromosome. XB105-16C was found to sporulate, and it in turn was dissected. The results are shown in Table 2. The monosomic segregates as a recessive lethal. As expected, centromere linkage is also demonstrated, since both viable spores in each ascus have the same genotype for *gal1* (as determined by complementation) in 10 of 11 two-spored asci. This is consistent with the previously reported 13.3% second-division segregation of *gal1* (3).

A total of 64 suspected monosomics were then isolated from 1,000 colonies of the sporulated X1221a tetraploid as described above. Only 29 of the 64 cultures sporulated. The remainder presumably were homozygous for one or the other of the mating-type alleles. Suspected monosomics for chromosomes I, II, V, VII, VIII, and for fragment 1 were sporulated and dissected. One of these carried *thr3* and *ura3* together on the presumably monosomic chromosome V, and all the viable spores from the sporulated isolate carried both *thr3* and *ura3* as expected.

Many of the 29 isolates that sporulated gave segregations of nonviable to viable spores that indicated that the isolate was a mixture of diploid

$$\frac{gal2 \ lys7 \ ade2 \ ade9 \ his8 \ MEL \ ma}{+ \ + \ + \ + \ his8 \ MEL \ ma} \times \frac{\alpha \ met1}{\alpha \ met1}$$

and monosomic cells. It was possible to isolate clones from these isolates which gave four viable spores from each ascus and were so judged to be diploid. Other clones originally monosomic apparently became mixtures of monosomic and diploid cells due to the faster growth of the diploid revertants, so that when sporulated they again gave some 2:2 and some 4:0 segregations for viable:nonviable spores. The diploid revertants were always homozygous for the marker that was used to indicate monosomy in the original isolate. For example, when the presumed monosomic for chromosome V carrying both *thr3* and *ura3* diploidized, the diploid was homozygous for both *thr3* and *ura3*. The number of asci with three or

TABLE 1. Tetrad analysis of the tetraploid XB105

Locus	No. of asci segregating				
	4:0 ^a	3:1	2:2	1:3	0:4
<i>leu1-1</i>	7	0	0	0	0
<i>ade1-1</i>	7	0	0	0	0
<i>asp5</i>	7	0	0	0	0
<i>trp1-1</i>	0	0	7	0	0
<i>ura^b</i>	2	1	4	0	0
<i>his2-1</i>	6	1 ^c	0	0	0

^a Spore colonies were scored for growth on synthetic complete media lacking the growth factors indicated. The ratio of prototrophic to auxotrophic spores per ascus is given.

^b Both *ura3* and *ura1* are in duplex condition in XB105.

^c The one spore that failed to grow on complete-histidine was designated XB105-16C. Only the seven complete asci are shown.

TABLE 2. *Tetrad analysis of the monosomic XB105-16C*

Locus	No. of asci segregating		
	2:0 ^a	1:1	0:2
<i>ade1-1</i>	11	0	0
<i>leu1-1</i>	11	0	0
<i>asp5</i>	11	0	0
<i>ura^b</i>	1	6	4
<i>his2-1</i>	0	0	11
<i>gall^c</i>	7	1	3

^a Prototrophs: auxotrophs.

^b Spores were not tested by complementation for *ura1* and *ura3*.

^c Spores were tested for complementation with haploid testers carrying *gall* in both mating types. Since *gal2* was also segregating, some of the spores scored "positive" above did not grow on galactose medium. None of the five one-spored asci gave exceptional spores. The spore viability was 42.1%.

four viable spores may not accurately represent the proportion of diploids in a population of diploid and monosomic cells, since the monosomic cells may sporulate with lower efficiency. In general, isolates presumed to be monosomic for the larger chromosomes or fragments grew poorly, sporulated poorly, and reverted more rapidly to the diploid condition. The most slowly growing isolate carried *leu1* and *ade6* in homo- or hemizygous condition. Both these markers are located on chromosome VII, the longest chromosome.

Two stable monosomics were isolated from among the 29 of 64 that would sporulate. These two, X1221a-7C and X1221a-3C, are both red on YEPD and thus carry *ade1* or *ade2* in homo- or hemizygous condition (1). All the spores from X1221a-3C and X1221a-7C failed to complement when crossed to *ade1* testers of both mating types (Table 3). Again, the missing chromosome segregates as a centromere-linked lethal: the centromere markers *leu1*, *arg4*, *trp1*, *ura3*, and α give nearly the expected second-division segregation frequencies (Table 4). These two strains are therefore monosomic for chromosome I, which carries the *ade1* marker. They remain monosomic after several transfers.

The stable monosomics so far isolated from tetraploid meiotic products in the manner described are monosomic for chromosomes I and VI. Strömnaes has obtained similar results by using DL-parafluorophenylalanine to induce chromosomal aberrations in diploids (6). The only probable monosomics he obtained were for chromosomes I and VI.

TABLE 3. *Tetrad analysis of the monosomics from X1221a*

Locus	X1221a-7C; no. of asci segregating			X1221a-3C; no. of asci segregating		
	2:0 ^a	1:1	0:2	2:0	1:1	0:2
<i>leu1</i>	6	0	6	9	0	0
<i>ade1^b</i>	0	0	12	0	0	9
<i>arg4</i>	6	2	4	4	3	2
<i>trp1</i>	7	0	5	6	0	3
<i>ura3</i>	12	0	0	4	1	4
<i>lys7</i>	12	0	0	9	0	0
αc	3	5	4	4	4	1

^a Prototrophs: auxotrophs.

^b All spores were *ade1*; none carried the *ade2* marker.

^c Mating-type locus, a: α .

TABLE 4. *Centromere linkage*

Centromere marker	Monosomic	Per cent second division segregation ^a	Per cent second division segregation expected ^b	<i>P^c</i>
<i>leu1</i>	X1221a-7C	0.0	4.9	.45
<i>arg4</i>	X1221a-7C	16.7	16.3	.99
<i>arg4</i>	X1221a-3C	33.3	16.8	.17
<i>trp1</i>	X1221a-7C	0.0	0.94	.75
<i>trp1</i>	X1221a-3C	0.0	0.94	.76
<i>ura3</i>	X1221a-7C	0.0	10.2	.28
<i>ura3</i>	X1221a-3C	11.1	10.2	.97
α	X1221a-7C	41.7	41.0	.99
α	X1221a-3C	44.4	41.0	.95
<i>gall</i>	XB105-16C	9.1	13.3	.74

^a The per cent second division segregation is calculated on the assumption that the monosomic segregates as a centromere-linked lethal, so that any 1:1 ascus represents a second-division segregation of a centromere-linked marker.

^b From Mortimer and Hawthorne (3).

^c *P* is the chi-square probability that the observed variation is due to chance.

DISCUSSION

The recovery of spores showing the recessive phenotype at loci triplex in the parent tetraploid is a convenient and relatively unambiguous method of isolating monosomics. Those isolates which show a 2:2 segregation of viable to non-viable spores must either be monosomic or heterozygous for a centromere-linked lethal and homozygous for the recessive marker used to identify them. Since the latter possibility requires two coincidental events with low individual spon-

taneous probabilities, it is a much less likely explanation. When chromosomes are multiply marked (as in the case of the monosomic for chromosome V cited above), any explanation other than that of monosomy involves postulating three or more independent spontaneous events. The *p*-fluorophenylalanine treatment of diploids results in a constellation of effects at high frequency whose interpretation is difficult, so that it is a less useful method for the isolation of monosomics (6).

Work is now in progress on the isolation of recombinationless mutants in monosomics and on the mapping of unlocated genes by mating haploids to monosomic spores.

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