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THE ISOLATION OF MONOSOMICS IN YEAST

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ABSTRACT

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/-). So 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 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: they are haploid cells that lack the chromosome that 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

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 *Saccharomyces 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: $\frac{a}{\alpha}$ $\frac{ade1\text{-}1}{+}$ $\frac{gal1}{+}$ $\frac{trp1\text{-}1}{+}$ $\frac{ura3}{ura3}$ $\frac{his2\text{-}1}{+}$ $\frac{leu1\text{-}1}{+}$ $\frac{arg4}{+}$ $\frac{met14}{+}$ $\frac{asp5}{+}$ $\frac{gal2}{+}$ $\frac{lys7}{+}$. Y02587 has the genotype $\frac{a}{a}$ $\frac{trp1\text{-}1}{trp1\text{-}1}$ $\frac{ura1}{ura1}$. The resulting tetraploid was triplex(A/A/A/a) at the loci adel (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 hours 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: $\frac{a}{\alpha}$ $\frac{adel}{+}$ $\frac{gal1}{+}$ $\frac{trp1}{+}$ $\frac{ura3}{+}$ $\frac{thr3}{+}$ $\frac{his2}{his2}$ $\frac{leu1}{+}$ $\frac{ade6}{+}$ $\frac{arg4}{+}$ $\frac{thr1}{+}$ $\frac{Cu}{cu}$ $\frac{gal2}{+}$ $\frac{lys7}{+}$ $\frac{ade2}{+}$ $\frac{ade9}{+}$ $\frac{his8}{his8}$ $\frac{mel}{Mel}$ $\frac{ma}{ma}$ X $\frac{\alpha}{\alpha}$ $\frac{met1}{met1}$. X1221a is triplex at the loci adel (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 X11), lys7 (chromosome XIII), and ade2 and ade9 (fragment1). This tetraploid was sporulated and the asci were treated with snail enzyme. The asci were disrupted [illegible]

parent cells plated out to a density of about 100 per Petri plate on yeast extract + peptone + dextose (YEPD). Cononies that failed to grow after replica-plating on minimal+methionine+histidine or on yeast extract+ 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 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 15.3% second division segregation of gal1 (3).

A total of 64 suspected monosomics were then isolated from 1000 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 non-viable to viable spores that indicated that the isolate was a mixture of diploid 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 [illegible] diploid cells due to the faster growth [illegible]

some 4:0 segregations for viable: non-viable 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 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, as indicated in Table 3. Again, the missing chromosome segregates as a centromere linked lethal: the centromere markers leu1, arg4, trp1, ura3, and a 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. Strømnaes has obtained similar results using DL-parafluorophenylalanine

to induce chromosomal aberrations in diploids (6). The only probable monosomics he obtained were for chromosomes I and VI.

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 inviable 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 whose individual spontaneous probabilities are low, 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 para-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.

Table I. Tetrad analysis of the tetraploid XB105

Locus	Number of asci segregating				
	4:0[a]	3:1	2:2	1:3	0:4
leu1-1	7	0	0	0	0
ade1-1	7	0	0	0	0
asp5	7	0	0	0	0
trp1-1	0	0	7	0	0
ura[b]	2	1	4	0	0
his2-1	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	Number of asci segregating		
	2:0[a]	1:1	0:2
ade1-1	11	0	0
leu1-1	11	0	0
asp5	11	0	0
ura[b]	1	6	4
his2-1	0	0	11
gal1[c]	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 gal1 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%

Table 3. Tetrad analysis of the monosomics from X1221a

Locus	X1221a-7C Number of asci segregating			X1221a-3C Number of asci segregating		
	2:0[a]	1:1	0:2	2:0[a]	1:1	0:2
leu1	6	0	6	9	0	0
ade1[b]	0	0	12	0	0	9
arg4	6	2	4	4	3	2
trp1	7	0	5	6	0	3
ura3	12 ~~8~~	0	~~4~~ 0	4	1	4
lys7	12	0	0	9	0	0
α[c]	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	%Second division segregation[a]	%Second division segregation expected[b]	P[c]
leu1	X1221a-7c	0.0	4.9	.45
arg4	"	16.7	16.8	.99
arg4	X1221a-3C	33.3	16.8	.17
trp1	X1221a-7C	0.0	0.94	.75
trp1	X1221a-3C	0.0	0.94	.76
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ura3	X1221a-3C	11.1	10.2	.97
α	X1221a-7C	41.7	41.0	.99
α	X1221a-3C	44.4	41.0	.95
gal1	XB105-16C	9.1	13.3	.74

[a]The percent 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.

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