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Genetic analysis of spontaneous half-sectored colonies of *Saccharomyces cerevisiae*

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SUMMARY

Genetic analysis of spontaneous half-sectored colonies of a diploid strain homozygous for *ade2* and heterozygous for linked markers *aro1A*, *hom2*, *ade8 and trp4* was undertaken. Some of these were due to an unexpectedly high rate of *ade2* reversion and others to a low incidence of haploid sectors. About 50% resulted from mitotic recombination. Only a few were due to mitotic crossing over alone, the majority resulted from non-reciprocal 'conversion', including recombination extending over two loosely-linked loci, and some colonies featured both mitotic crossing over and mitotic conversion. The latter indicate that negative interference operates for mitotic recombination. The rate of mitotic recombination for *ade8*, calculated from the frequency of half-sectored colonies was $3{\cdot}1 \times 10^{-4}$ per cell division.

1. INTRODUCTION

Several studies have been made of radiation-induced mitotic segregation in yeast. Most authors have concluded that the principal mechanism of induced intergenic recombination is mitotic crossing over (James & Lee-Whiting, 1955; Johnston, 1962; Esposito, 1968; Nakai & Mortimer, 1969), although a lower frequency of non-reciprocal mitotic recombination (Roman & Jacob, 1958) has also generally been observed. In addition, Wilkie & Lewis (1963) favoured the process of meiotic centromere disjunction followed by restitution of diploidy and Hurst & Fogel (1964) proposed the mechanism of 'parameiosis'. There has, however, been no extension of these analyses to mitotic recombinants occurring spontaneously. Results of such an extension are reported below.

2. MATERIALS

(i) *Strain*

A diploid hybrid of *S. cerevisiae*, X190, was synthesized from strains originally received from Dr R. K. Mortimer, Berkeley. It had the genotype

$$\frac{a}{\alpha}\ \frac{ade2}{ade2}\ \frac{aro1A}{+}\ \frac{hom2}{+}\ \frac{ade8}{+}\ \frac{trp4}{+}\ \frac{ura3}{+}\ \frac{lys7}{+}.$$

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(ii) *Media*

Broth was YEPAD: 1% Difco yeast extract, 2% Difco peptone, 2% dextrose, 20 mg/l additional adenine. Plates for obtaining half-sectored colonies contained modified YEPAD: 1% yeast extract, 0·5% peptone, 0·5% dextrose, 20 mg/l adenine, 2% agar. Plates for scoring nutritional and fermentation markers were as described by Hawthorne & Mortimer (1960). Presporulation growth medium was GNA: Difco nutrient agar 2·3%, yeast extract 1%, dextrose 5%, additional agar 0·5%. Sporulation medium was either RA: 0·3% sodium acetate, 0·02% raffinose, 2% agar; or PA: 1% potassium acetate, 0·25% yeast extract, 2% agar.

3. METHODS

Broth contained in Erlenmeyer flasks was inoculated with cells of 2- to 3-day-old pink colonies. After 3 days incubation on a rotary shaker at 30 °C, cells were washed and diluted in sterile distilled water. Approximately 150 cells were plated for detection of half-sectored colonies. Plates were screened after 5–6 days incubation at 30 °C.

Colonies of strain X 190 are normally red because of the *ade2* mutation. White sectors may be of two types: (*a*) adenine-independent due to *ade2* reversion, or (*b*) adenine-dependent because of homozygosis for *ade8* (Roman, 1956). The supplement of adenine allowed red and white half-sectors to be virtually identical in size.

Tetrad analysis was performed by ascus wall digestion (Johnston & Mortimer, 1959), micromanipulation of ascospores, culturing of spore colonies and replica-plating.

4. RESULTS

Of a total of 61 295 colonies of strain X 190, 36 were clearly half-sectored for colour. In addition, 20 colonies contained white sectors comprising about one-third of the colony. Some of these may represent first-division events but with a reduced growth rate of the white sector. Since they equally well may result from second-division events with faster growth of the white sector, they were excluded from further analysis.

Red and white half-sectors were tested for their phenotypes. Unexpectedly, 13 of the 36 white sectors were adenine-independent and therefore due to *ade2* reversion. Both half-sectors of four of these colonies were subjected to tetrad analysis and, in all cases, both sectors were heterozygous for *ade8*, *trp4*, *hom2* and *aro1A*. This result gives a reversion frequency for *ade2* of approximately 2×10^{-4} per generation for the first-division of plated cells. This high rate was not found for cells cultured in liquid medium (Thornton & Johnston, 1971). Of the remaining 23 colonies, 8 sectored for only *ade8*, 13 sectored for *ade8* and *trp4*, 1 sectored for *ade8*, *trp4*, *hom2* and *aro1A*, and 1 sectored for *ade8* but was tryptophan-dependent in both sectors.

Cells of both sectors of 3 of these 23 colonies could not be induced to sporulate. These sectors were mated to tester haploid strains and the resulting crosses ana-

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lysed. All three white half-sectors proved to be haploid, of genotype *ade2 ade8*, but the three crosses of red sectors would not sporulate. The red sector of another 1 colony also would not sporulate, although the complementary white sector did so. In this case, further analysis proved that this red sector was haploid, and the white sector diploid. Thus, three white half-sectors result from a process of haploidy, corresponding to a frequency of approximately 5×10^{-5} per cell division, and, in one more colony, although the white sector is diploid, the red sector is haploid. These haploid sectors most likely arise from a very low level of asci present in the plated cultures, though no asci were detected by microscopic examination. No process analogous to haploidy by successive non-disjunctions in *Aspergillus* (Pontecorvo & Käfer, 1958) has been found in yeast.

Table 1. *Genotypes of 18 spontaneous half-sectored colonies of strain X 190*

(No. of colonies showing reciprocal or non-reciprocal sectoring.)

Phenotype	Reciprocal	Non-reciprocal	Reciprocal and Non-reciprocal
R ade + W ade +	+ trp R + + W ade trp ade + – 1	ade trp R + + W ade trp ade + – 5	
R ade + W ade trp	+ + R + + W ade trp ade trp – 2	ade trp R + + W ade trp ade trp – 5	+ trp R + + W ade trp ade trp – 5

R = red, W = white. For convenience, ade and trp are used to denote genes *ade8* and *trp4* respectively.

The 1 colony which was tryptophan-dependent in both sectors was diploid and showed reciprocal homozygosis for the *ade8* locus. The genotypes of the remaining 18 colonies, those which are completely diploid and sector for either *ade8* alone, or both *ade8* and *trp4*, are shown in Table 1. That only one colony of six sectoring for *ade8* alone shows reciprocality suggests that there is a higher rate of spontaneous gene-conversion than of mitotic crossing over when segregation is for only one locus. The results of Nakai & Mortimer (1969), Johnston (1962) and Esposito (1968) show that there are approximately equal frequencies of mitotic crossing over (single locus segregation) and conversion induced by X-rays and ultraviolet light. Thus it appears that mitotic crossing over is increased more by radiation than is conversion.

Of the 12 colonies sectoring for both *ade8* and *trp4*, only two had reciprocal genotypes for both loci in red and white sectors. Five colonies showed non-reciprocal recombination for both loci and another five were reciprocal for *ade8* and non-reciprocal for *trp4*. Thus, mitotic crossing over alone appears to produce only about 20% of these colonies, while about 40% arise from 'conversion' and around 40% seem to result from both mitotic crossing over (*ade8*) and conversion

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(*trp4*). However, 'conversion' for both *ade8* and *trp4* extends to both loci, which are only loosely linked, and therefore does not seem an identical process to orthodox gene conversion. The frequency of these colonies is too high for them to be satisfactorily explained by simultaneous, independent conversions at both loci. Neither does it seem likely that mitotic crossing over for *ade8* and conversion for *trp4* can be independent events. Since *trp4* is distal to *ade8* (R. K. Mortimer, personal communication), and therefore two cross-overs are required to produce those colonies showing reciprocal recombination for only *ade8*, it appears that intergenic negative interference operates for this chromosomal region. A similar type of phenomenon, whereby one event makes occurrence of a second event in a neighbouring chromosomal region more likely, appears to operate for mitotic crossing over proximal to the *ade8* locus and non-reciprocal recombination for *trp4*. High negative interference has been observed for mitotic recombination in *Aspergillus* (Pritchard, 1955), but in this case the events were intragenic.

The frequency of 19 half-sectored colonies, which were diploid and not due to *ade2* reversion, gives a rate of mitotic recombination for *ade8* of $3{\cdot}1 \times 10^{-4}$ per cell division. This agrees closely with the rate of $3{\cdot}4 \times 10^{-4}$ per cell generation obtained by growth in liquid medium (Thornton & Johnston, 1971). The rate calculated from the frequency of half-sectors is, however, subject to inaccuracy due to the possibility that some one-third and one-quarter sectored colonies may result from first-division mitotic segregation, the latter in budded cells.

5. DISCUSSION

Mitotic crossing over has been proposed as the principal mechanism underlying radiation-induced and chemically induced mitotic recombination in yeast (Nakai & Mortimer, 1969; Zimmermann, Schwaier & Laer, 1966). However, when sectoring is for a single locus, only around 50% of radiation-induced recombination is due to mitotic crossing over, while an approximately equal frequency is non-reciprocal and possibly due to gene conversion (Johnston, 1962; Esposito, 1968; Nakai & Mortimer, 1969). In some cases, however, 'non-reciprocal recombination' may simply result from induced mutation of a wild-type allele of *ade2* at the four-strand stage. On the other hand, Wildenberg (1970) has concluded that both X-ray-induced reciprocal and non-reciprocal mitotic recombination in yeast can occur at the two-strand stage.

In contrast to induced half-sectored colonies, analysis of those arising spontaneously indicates that a minority are due to mitotic crossing over. When segregation is for *ade8* alone, the ratio of conversion to mitotic crossing over is 5:1, although statistical accuracy is low. In this case, non-reciprocal recombinants do not seem due simply to forward mutation, for the spontaneous mutation rate would be approximately 8×10^{-5} per division (5/61 295). No comparable mutation rate has been observed in haploid *ade2* mutants for the overall mutation to white, adenine double mutants, i.e. *ade2 adex*, where $x = 3, 4, \ldots, 9$ (Johnston, unpublished results). Furthermore, on plating cells of two of the white half-sectors

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of these colonies showing non-reciprocal recombination, no red colonies were detected among a total of 32788 colonies. This suggests that the *ade8* genes of these sectors are homoalleles, since a new *ade8* mutation would likely be heteroallelic to that present in strain X 190 and the heteroallelic diploid would probably produce wild-type intragenic recombinants.

Of those colonies sectoring for both *ade8* and *trp4*, about 60% show reciprocal recombination for *ade8* but only about 20% display reciprocality for *trp4*. Of most interest are the five colonies showing non-reciprocal recombination for both *ade8* and *trp4*. This coincident non-reciprocal recombination for two widely separated loci is different from coincident conversion of two mutant sites (Fogel & Mortimer, 1969) and cannot be explained by recently proposed molecular models of gene conversion (Stahl, 1969; Fogel & Mortimer, 1970; Fincham & Holliday, 1970).

These models of intragenic, meiotic gene conversion may not be applicable to mitotic recombination and to selection of mitotic recombinants due to intergenic events. Any interpretation of these coincident non-reciprocal recombinants must be cautious, however, for there is no proof that these five colonies are necessarily the result of first-division events. They could be explained by a combination of mitotic crossing over at the second post-plating division and death of the complementary daughter cell, but only if faster growth allows the surviving recombinant cell to form a half-sector. In this case, the red half-sector is of parental genotype and has not been associated with an early recombinational event.

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