THE EFFECT OF THE MATING-TYPE ALLELES ON
INTRAGENIC RECOMBINATION IN YEAST

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THE alleles a and α govern the mating reaction in Saccharomyces cerevisiae
(LINDEGREN and LINDEGREN 1943). High-frequency mating is obtained when
haploid a or diploid a a cells are mixed with α or a α cells (ROMAN, PHILLIPS,
and SANDS 1955). Matings of cells of like mating type are rare and have been
observed, in fact, only between a cells. The mating type alleles also govern sporulation. Cells that have at least one a and one α allele can be induced to sporulate,
whereas a a and α α cells do not sporulate under any of the conditions that have
been tried.

Intragenic recombination occurs spontaneously in a α diploids and its frequency
can be increased substantially with ultraviolet light (ROMAN and JACOB 1957).
Thus there is reason to believe that chromosomal pairing can occur during mitosis
in cells of this type (see also WILKIE and LEWIS 1963; HURST and FOGEL 1964).
The fact that a a and a α cells do not sporulate suggests the possibility that such
cells are defective in the meiotic process. Mitotic intragenic recombination should
not be affected if the lesion does not interfere with chromosomal pairing and
exchange. If the lesion prevents or inhibits pairing and exchange, a reduction in
intragenic recombination would be expected. The experiments described below
were designed to test these alternatives.

MATERIALS AND METHODS

The diploid cultures used in these experiments were derived from strains maintained in the
Department of Genetics, University of Washington, Seattle. Diploids heterozygous for the mating
type alleles were constructed in two ways: (1) by mating haploid cells of opposite mating type
and selecting for the resulting diploid (C18+ and C187); and (2) by isolating with a micro-
manipulator the diploid segregants (11-5B, 11-35A, 54-8C) from sporulated tetraploid cultures
of genotype a a a a (ROMAN, PHILLIPS, and SANDS 1955). The tetraploid cultures were obtained
from matings of a a × a a diploids.

Diploids of genotypes a a (11-5D and 54-8B) and a α (11-5C) were also derived as segregants
from tetraploid cultures. In addition, a a cells (N3 and N7) were obtained by mixing two haploid
a strains that were complementing auxotrophs for other markers and plating for prototrophs on
medium lacking the respective requirements. N3 and N7 were the a α parents of the tetraploids
in the 11 and 54 series, respectively.

Intragenic recombination was measured by the frequency of reversion to prototrophy in

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heteroallelic combinations (ROMAN 1956) of the adenine alleles $ad_{b,3}$, $ad_{b,4}$, and $ad_{b,6}$. Two combinations were utilized: $ad_{b,3}/ad_{b,4}$ and $ad_{b,3}/ad_{b,6}$. The cells were grown aerobically for 48 hrs (to stationary phase) at 30°C, in a medium containing 1% yeast extract, 2% peptone, and 2% glucose, and supplemented with 75 mg/l of adenine sulfate to minimize the selective advantage of prototrophs arising during the growth of the culture. Spontaneous revertants to adenine independence were assayed by plating $10^6$ cells per plate on synthetic adenineless medium. For ultraviolet induction of intragenic recombination, $10^6$ cells were plated on individual plates of adenineless medium and irradiated for 30 sec with a germicidal lamp that emitted mainly 2537Å. The output of the lamp was 16 ergs/mm²/sec at a distance of 14 inches from the lamp. Survival was measured by irradiation of cells plated on complete medium. The operations were performed under yellow light to avoid photoreactivation.

Since diploids homozygous for the mating-type alleles, $a a$ or $a a$, could exhibit a reduced frequency of reversion to adenine independence either because they are homoallelic for the $ad_1$ alleles or homozygous for mating type, it was important to verify that they were heteroallelic. The verification procedure can be illustrated as follows: An $a a$ segregant, presumed to be $ad_{b,3}/ad_{b,4}$, was crossed with an $a a ad_{b,3}/ad_{b,3}$ (or $ad_{b,3}/ad_{b,3}$) diploid derived from a mating of two $a$ strains and therefore of known genotype. Asci from the tetraploid were dissected and the segregants were tested further in those cases in which all four segregants from an ascus were $a a$. The four segregants were sporulated and plated for the frequency of revertants to adenine independence. When the $a a$ diploid was heteroallelic, two of the segregants gave the high frequency of revertants expected from the heteroallelic combination (ROMAN 1956). The other two gave no revertants in some $10^5$ asci plated.

RESULTS AND DISCUSSION

The results of the treatments of the $a a$, $a a$, and $a a$ diploids are given in Table 1. Each frequency represents the counts of 10 plates, $10^6$ cells per plate, expressed as the number of induced prototrophs per $10^6$ surviving cells. It is clear that the same heteroallelic combination gives substantially different recombination responses depending on whether the mating-type locus is in homozygous or heterozygous condition. Within each heteroallelic combination, the higher frequencies are regularly associated with the heterozygous $a a$ condition.

Among the mating-type homozygotes, those derived from the dissection of

<table>
<thead>
<tr>
<th>Culture number</th>
<th>Genotype</th>
<th>Percent survival</th>
<th>Pre-existing Prototrophs/$10^6$ cells</th>
<th>Induced Prototrophs/$10^6$ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 187</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>96</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>11-5B</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>94</td>
<td>5</td>
<td>116</td>
</tr>
<tr>
<td>11-35A</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>94</td>
<td>20</td>
<td>118</td>
</tr>
<tr>
<td>11-5D</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>96</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11-35C</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>96</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>N3</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>97</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>C 184</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>99</td>
<td>4</td>
<td>149</td>
</tr>
<tr>
<td>54-8C</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>100</td>
<td>6</td>
<td>144</td>
</tr>
<tr>
<td>54-8B</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>100</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>N7</td>
<td>$a a ad_{b,3}/ad_{b,4}$</td>
<td>95</td>
<td>4</td>
<td>75</td>
</tr>
</tbody>
</table>
tetraploid cells (the 11 and 54 series) are distinctly lower in recombination frequency than those obtained directly from the matings of $a \times a$ cells (N3 and N7), especially when the comparison is made within the respective heteroallelic combinations. The variation may reflect a difference in genetic background, i.e., in recombination modifiers other than $a$ and $a$. It is, however, possible that the method of obtaining the diploids is itself important in determining the level of recombination. The rare $a \times a$ matings may represent fusions of an $a$ cell with a cell that is genotypically $a$ but sufficiently $a$-like in phenotype to permit a mating. The resulting diploids might therefore resemble $a a$ diploids in their recombinational response to ultraviolet light. Our results could be explained if this metastable state were confined to mitotically dividing cells. More extensive tests are required before a decision can be reached between these alternatives.

To test the effect of ultraviolet light on recombination in another non-sporulating variant in yeast, we chose a respiratory-deficient cytoplasmic mutant strain of the type described by Ephrussi (1953). This diploid, C200P, had the genotype $a a a_d b / a_d d$ and was therefore like C184 and 54–8C in Table 1 in this respect. The results with C200P and with the normal progenitor strain C200G, shown in Table 2, indicate that the lesion affecting sporulation in the respiratory-deficient strain does not affect recombination. In this case, therefore, chromosomal pairing and exchange appear to be unimpaired.

The results presented above implicate the $a$ and $a$ alleles as regulators that specify the mating reaction, affect recombination, and govern sporulation. The nature of the block to recombination in $a a$ and $a a$ diploids remains unknown as does the complementation product that would be expected in $a a$ diploids (see Gutz 1967, for evidence for a complementation product for sporulation in Schizosaccharomyces pombe). It is noteworthy that $a a$ and $a a$ cells are no more sensitive to kill by ultraviolet light than $a a$ cells (Laskowski 1962); the latter are, however, more resistant to ionizing radiation. The results indicate further that the induction of recombination by ultraviolet light is not simply a matter of the induction of chromosomal pairing by this agent (Holliday 1961). If ultraviolet light does indeed induce chromosomal pairing, it can do so only in the appropriate genotype. Finally, as the comparison between the mating-type homozygotes and the respiratory-deficient mutant shows, recombination can be used as a convenient

<table>
<thead>
<tr>
<th>Culture number</th>
<th>Dose</th>
<th>Survival</th>
<th>Pre-existing Prototrophs/10^6 cells</th>
<th>Induced Prototrophs/10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>C200G (normal)</td>
<td>30&quot;</td>
<td>100%</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>60&quot;</td>
<td>84%</td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td>C200P (deficient)</td>
<td>30&quot;</td>
<td>82%</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>60&quot;</td>
<td>83%</td>
<td>10</td>
<td>139</td>
</tr>
</tbody>
</table>
indicator to separate non-sporulating mutants into two broad classes, early mutants that affect both recombination and sporulation, and late mutants that affect sporulation alone.

SUMMARY

Diploid yeast cells that are incapable of sporulation because they are homozygous for the mating-type alleles $a$ and $a$ show a marked reduction in ultraviolet-induced intragenic recombination in comparison with cells that are heterozygous $a a$ and capable of sporulation. Cells that are $a a$ but are incapable of sporulation because they are respiratory-deficient, are normal in their response to ultraviolet light.

LITERATURE CITED


