GENETIC ANALYSIS OF GAMMA-RAY MUTAGENESIS IN YEAST.
II. ALLELE-SPECIFIC CONTROL OF MUTAGENESIS

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ABSTRACT

We find that partially different sets of gene functions are required for the production of different kinds of mutations induced by $^{60}$Co $\gamma$ rays in Saccharomyces cerevisiae. This observation is very similar to others made previously with respect to UV mutagenesis (Lawrence and Christensen 1978a,b, 1979) and confirms the conclusion that such distinctive patterns of genetic control reflect properties of the test alleles and their genetic locations, rather than the kinds of lesions required to revert them. The data also support the model of mutagenic repair outlined in the first paper of this series (McKee and Lawrence 1979), in which partially different sets of gene functions are required for the production of different kinds of mutations, the formation of mutations at different genetic sites and the induction of mutations by different mutagens.

In work reported previously (McKee and Lawrence 1979), the genetic control of ionizing-radiation-induced mutagenesis in Saccharomyces cerevisiae was investigated by measuring the reversion frequency of cycl-9, an ochre allele of the structural gene for iso-1-cytochrome c (Stewart et al. 1972) in a variety of radiation-sensitive strains. Use of only a single test allele, such as cycl-9, fails to reveal all aspects of the genetic control, at least when UV is employed as mutagen, because it has been found (Lawrence and Christensen 1978a,b, 1979) that the production of different mutational events leading to the reversion of other cycl test alleles depends on the activity of partially different sets of genes.

On the basis of such data, the set of 16 cycl test alleles used could be divided into five categories; the proline missense mutation cycl-115 and the initiation mutation cycl-131; the highly UV-revertible ochre allele, cycl-9; the remaining ochre, amber, initiation and missense alleles; the frameshift allele, cycl-31; and the remaining frameshift alleles. Although not understood in all cases, the differentiation among these five groups seems to depend on the genetic nature of the mutations and the sites that are altered, not on the type of premutation lesion required to revert the alleles. With the exception of cycl-31, which was not examined, UV-induced reversion of alleles representative of each of these groups was substantially and more-or-less equally reduced by photoreactivation in both excision-proficient and deficient backgrounds, implying that the majority of revertants in all categories were caused by cyclobutyl pyrimidine dimers.
If the differentiation between these groups of test alleles does in fact reflect their genetic rather than their photochemical properties, it might also be found when other mutagens are used. We have, therefore, examined the gamma-ray-induced reversion frequency of several representative cyc1 alleles in diploid strains homozygous for rev1, rev2, rev3 or rad6 mutations, in most instances the same strains used previously in the UV experiments. We find that partially different sets of gene functions are also required for the production of gamma-ray-induced reversion of the various alleles examined, a result that supports the view that differentiation among the various categories of alleles does not depend solely on differences in the kinds of premutational lesions required to revert them. We also find a number of significant contrasts between the UV and γ-ray data, showing that the type of lesion has some influence, even though a minor one. Overall, these results provide support for the model of mutagenic repair or recovery outlined in the first paper of this series (McKee and Lawrence 1979) in which it is suggested that partially independent sets of gene functions are required for the production of mutations of different kinds, for their formation at different sites and for their induction by different mutagens.

MATERIALS AND METHODS

Strains: The four cyc1 test alleles used in this work were the ochre mutation, cyc1-9; the proline missense mutation, cyc1-115; the initiation mutation, cyc1-131; and the frameshift mutation, cyc1-183. The positions and types of base-pair changes in these mutations are listed in Table 1, together with references to the original papers from which further information can be obtained. Gamma-ray-inducer reversion of these alleles (excluding cyc1-9) was studied in diploid strains homozygous for rev1-1, rev2-1 or rev3-1, the same strains that were used in the earlier UV experiments (Lawrence and Christensen 1978a,b, 1979). The reversion of cyc1-9 and arg4-17 was examined in specially constructed diploid strains homozygous for these alleles, and the same was true of the rad6 series of diploids.

Media and methods: Reversion frequencies of arg4-17 and the cyc1 alleles were determined by growing strains in yeast-extract peptone dextrose medium for three days, irradiating suspensions of washed cells in water, and plating appropriate dilution onto semi-synthetic lactate medium for cyc1 reversion and onto similar medium supplemented with arginine for corresponding estimates of viability. A more complete description of the media used, the irradiation source and its dosimetry and the general experimental procedure can be found in Lawrence and Christensen (1976; 1978a) and McKee and Lawrence (1979). Between ten and 100 plates were used for each dose and dilution for scoring cyc1 revertants and four plates for viability estimates.

TABLE 1

<table>
<thead>
<tr>
<th>Type</th>
<th>cyc1 allele</th>
<th>Number</th>
<th>Mutant codon</th>
<th>Normal codon and position</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation mutant</td>
<td>131</td>
<td>GUG</td>
<td>AUG</td>
<td>-1</td>
<td>Stewart et al. (1971)</td>
</tr>
<tr>
<td>Proline missense mutant</td>
<td>115</td>
<td>CCPy</td>
<td>CUPy</td>
<td>14</td>
<td>J. W. Stewart and F. Sherman (personal communication)</td>
</tr>
<tr>
<td>Frameshift mutant</td>
<td>183</td>
<td>+A</td>
<td>AAA</td>
<td>10</td>
<td>Sherman and Stewart (1973)</td>
</tr>
<tr>
<td>Ochre mutant</td>
<td>9</td>
<td>UAA</td>
<td>GAA</td>
<td>2</td>
<td>Stewart and Sherman (1974)</td>
</tr>
</tbody>
</table>

The type and position of nucleotide alterations in the various cyc1 alleles studied
TABLE 2

*Induced reversion frequency of arg4–17 and various cyc1 alleles in diploid strains homozygous or heterozygous for rev1–1, rev2–1 or rev3–1 exposed to 25 or 50 Krad of 60Co γ rays*

<table>
<thead>
<tr>
<th>Strain</th>
<th>25 Krad</th>
<th>50 Krad</th>
<th>25 Krad</th>
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<th>25 Krad</th>
<th>50 Krad</th>
<th>25 Krad</th>
<th>50 Krad</th>
<th>25 Krad</th>
<th>50 Krad</th>
</tr>
</thead>
<tbody>
<tr>
<td>rev1–1/rev1–1</td>
<td>79 (61)</td>
<td>292 (14)</td>
<td>30 (29)</td>
<td>117 (4)</td>
<td>59 (43)</td>
<td>285 (7)</td>
<td>13 (89)</td>
<td>26 (58)</td>
<td>32 (76)</td>
<td>31 (35)</td>
</tr>
<tr>
<td>rev1–1/ +</td>
<td>165 (54)</td>
<td>320 (25)</td>
<td>68 (71)</td>
<td>198 (23)</td>
<td>34 (74)</td>
<td>48 (51)</td>
<td>145 (100)†</td>
<td>243 (83)†</td>
<td>168 (130)*</td>
<td>357 (71)*</td>
</tr>
<tr>
<td>rev2–1/rev2–1</td>
<td>51 (62)</td>
<td>190 (14)</td>
<td>115 (63)</td>
<td>374 (20)</td>
<td>24 (59)</td>
<td>60 (14)</td>
<td>26 (64)</td>
<td>45 (76)</td>
<td>27 (108)</td>
<td>41 (60)</td>
</tr>
<tr>
<td>rev2–1/ +</td>
<td>64 (56)</td>
<td>367 (8)</td>
<td>98 (56)</td>
<td>251 (19)</td>
<td>17 (83)</td>
<td>39 (51)</td>
<td>145 (100)†</td>
<td>243 (83)†</td>
<td>187 (100)*</td>
<td>379 (64)*</td>
</tr>
<tr>
<td>rev3–1/rev3–1</td>
<td>14 (72)</td>
<td>58 (15)</td>
<td>12 (64)</td>
<td>24 (26)</td>
<td>11 (64)</td>
<td>38 (28)</td>
<td>- - - -</td>
<td>- - - -</td>
<td>5 (64)</td>
<td>26 (20)</td>
</tr>
<tr>
<td>rev3–1/ +</td>
<td>120 (69)</td>
<td>334 (27)</td>
<td>86 (64)</td>
<td>577 (27)</td>
<td>35 (86)</td>
<td>74 (49)</td>
<td>176 (94)</td>
<td>301 (67)</td>
<td>221 (100)</td>
<td>288 (63)</td>
</tr>
</tbody>
</table>

All entries in the table are the average of two replicate strains except where indicated.

* Data from one strain.
† Average of eight strains (data from McKee and Lawrence 1979).
RESULTS

The data given in Table 2 show that although the γ-ray-induced reversion frequency of some of the cyc1 test alleles is much reduced in rev1, rev2 or rev3 mutant strains, other alleles revert at frequencies that are close to normal in these strains. The pattern of dependence of the different sets of mutation events on the REV1, REV2 and REV3 gene functions is quite similar to that seen for UV mutagenesis in most cases, but significant differences are also found.

The reversion frequency of cyc1-115, cyc1-131 and cyc1-183 is approximately normal in rev1 mutant strains, but that of cyc1-9 and arg4-17 is only about one-tenth that of the wild type. This pattern is very similar to that found for UV-induced reversion in these strains (Lawrence and Christensen 1978a) although it is possible, in contrast to the UV results, that the reversion frequency of cyc1-115 and cyc1-131 in rev1 strains is only one-half that of the wild-type controls; the error variation, however, is too large to prove this point. The use of previous results for cyc1-9 reversion as the wild-type control is appropriate since the rev1 strains were constructed from pedigrees with closely related genetic backgrounds.

The rev2-1 mutation has no influence on the reversion frequency of cyc1-115, cyc1-131 or cyc1-183 induced by γ-rays, but it reduces the incidence of cyc1-9 and arg4-17 reversion by factors of five and about eight, respectively (Table 2), a result that is very similar, both qualitatively and quantitatively, to that found previously using W (Lawrence and Christensen 1978b).

Finally, the influence of the rev3-1 mutation on the reversion of the test alleles induced by the two radiations is also similar, the frequencies being reduced in all cases, but in this case the relative sizes of the reductions varies among the alleles in a significantly different pattern with UV and γ rays. The γ-ray-induced reversion frequencies of cyc1-115 and cyc1-131 are only 10 to 20% of the corresponding wild-type values in rev3 strains (Table 2), compared to 50% or more with UV (Lawrence and Christensen 1979), while the estimates for cyc1-183 are 30 to 50% using γ rays (Table 2) and less than 10% using UV. The reversion frequencies of cyc1-9 and arg4-17 in rev3 strains are less than 10% of the control frequency with either kind of radiation.

Although the mutational events induced by γ rays that revert the various test alleles show varying dependence on the REV1, REV2 and REV3 gene functions, and in many instances are completely independent of them, they are nevertheless all completely dependent on RAD6 function and, therefore, a consequence of error-prone recovery. As shown in Table 3, the reversion frequency of all of the cyc1 alleles is much reduced in diploid strains homozygous for rad6-1, and in fact, although the γ-ray doses used induced significant numbers of revertants in the wild-type controls, these doses failed to increase the reversion frequency significantly above spontaneous levels in all of the rad6 mutants.

As pointed out previously (Lawrence and Christensen 1978a), the pattern of dependence of cyc1 test allele reversion on the function of RAD or REV genes is an inherent property of these alleles, not a characteristic of the particular strains
## TABLE 3

*Induced reversion frequency of various *cyc1* alleles in diploid strains homozygous or heterozygous for *rad6* exposed to 5 and 10 krad of $^{60}$Co $\gamma$ rays*

<table>
<thead>
<tr>
<th>Strain</th>
<th><em>cyc1-I</em></th>
<th><em>cyc1-I</em></th>
<th><em>cyc1-I</em></th>
<th><em>cyc1</em>I</th>
<th><em>cyc1-I</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 Krad</td>
<td>10 Krad</td>
<td>5 Krad</td>
<td>10 Krad</td>
<td>5 Krad</td>
</tr>
<tr>
<td><em>rad6-1/rad6-1</em></td>
<td>0.7 (73)</td>
<td>0.1 (55)</td>
<td>0.7 (87)</td>
<td>1.3 (78)</td>
<td>0.8 (59)</td>
</tr>
<tr>
<td><em>rad6-1/+</em></td>
<td>10.0 (89)</td>
<td>12.4 (100)</td>
<td>13.1 (102)</td>
<td>33.0 (78)</td>
<td>3.3 (104)</td>
</tr>
</tbody>
</table>

All entries in the table are the average of two replicate strains except where indicated.

* Average of eight strains (data from McKee and Lawrence 1979).
used or the consequence of mutational events outside the \textit{cyc}1 locus. There is no
evidence, for example, that \textit{cyc}1--115 or \textit{cyc}1--131 diploids carry suppressors of
the \textit{rev}1--1 or \textit{rev}2--1 alleles since the sensitivity of these strains, as well as the
induced frequency of \textit{arg}4--17 reversion, is quite typical of all other strains. Similarly,
virtually all revertants scored were the consequence of mutations within the
\textit{cyc}1 locus. Translational or nontranslational suppressors are rarely found
under the experimental conditions used, and their absence was confirmed by
spectroscopic examination of a sample of revertant clones.

\textbf{DISCUSSION}

Previous work (LAWRENCE and CHRISTENSEN 1978a,b, 1979), in which the
frequency of UV-induced reversion of a variety of contrasting \textit{cyc}1 alleles was
measured in \textit{rev}1, \textit{rev}2 and \textit{rev}3 mutant strains, showed that \textit{RAD}6-dependent
mutagenic repair is not a single process, but rather comprises a number of partial-
ly independent processes, each of which gives rise to a different category of
mutations. It was concluded that differentiation among these categories depends
on the properties of the \textit{cyc}1 alleles themselves and their genetic location, rather
than on differences in the types of casual premutational lesions, since cyclobutane-
type pyrimidine dimers appear to be responsible for the production of most mu-
tations of each type (LAWRENCE and CHRISTENSEN 1978a). The less extensive
data, given in Table 2 of the present paper, concerning \textit{60}Co \textit{\gamma}--ray-induced rever-
sion of \textit{cyc}1 alleles support this conclusion; although the two radiations are likely
to produce different kinds of premutational lesions, the patterns of reversion of the
\textit{cyc}1 alleles induced by two rations in the \textit{rev} mutant strains were very similar.
The data in Table 2 also confirm the existence of several \textit{RAD}6-dependent muta-
genic processes for lesions other than those produced by UV.

Although it is likely that differentiation among the various categories of muta-
tional events depends on the genetic properties of the \textit{cyc}1 alleles themselves and
their locations within the gene, it has not always been possible to identify the
crucial features that are responsible for the discrimination, despite almost com-
plete information concerning the base-pair changes responsible for reversion on
the surrounding nucleotide sequence (see Table 1 for references). As might be
expected, base-pair substitutions and addition/deletion mutations fall into differ-
ent categories, the \textit{REV}1 gene function being necessary for the production of the
former but not the latter, for example; but the basis for the discrimination be-
tween the typical base-pair substitution alleles \textit{cyc}1--115 and \textit{cyc}1--131 is not yet
evident. It does not depend on the type of base-pair substitution required to revert
these alleles, nor on the particular site at which substitution occurs (LAWRENCE
and CHRISTENSEN 1978a). It appears that features other than nucleotide sequence
may be responsible, such as the modification of bases, the presence of DNA-
binding molecules or the quaternary structure of the chromatin.

The relative reversion frequency of the various \textit{cyc}1 alleles in \textit{rev} mutant
strains is qualitatively very similar with either radiation as mutagen, but quan-
titative differences can also be seen. Relative to the wild-type frequency, the \textit{\gamma}--ray-induced reversion frequency of \textit{cyc}1--115 and \textit{cyc}1--131 is reduced by a fac-
tor of about seven in \textit{rev}3 strains, although the factor is only two when UV is
used. Conversely, the γ-ray-induced reversion frequency of the frameshift allele cycl-183 is reduced by a factor of only about four in such strains, while the factor is over ten for UV-induced reversion. There results suggest that the type of pre-mutational lesion responsible for mutagenesis plays at least some role in the discrimination between mutational categories, a conclusion supported by the data of PRAKASH (1976); the reversion frequency of cycl-131 induced by the UV-like chemical mutagen 4-nitroquinoline oxide is much reduced in rev1, rev2 and rev3 mutant diploids, although UV-induced reversion is not (LAWRENCE and CHRISTENSEN 1976, 1978a,b, 1979).

The results given in Table 2, together with those in earlier papers, support the model of RAD6-dependent repair outlined in the first paper of this series (McKee and LAWRENCE 1979). We propose that RAD6-dependent repair is composed of both error-free and error-prone processes, and that the error-prone processes are further subdivided according to type of mutation produced, site of mutation and type of mutagen. Besides accommodating the kind of data given in Table 2, such a model also explains why what appears at first sight to be a single mutagenic pathway for repair or recovery is capable of coping with a large variety of contrasting premutational lesions.

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LITERATURE CITED


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