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# Metabolic Suppressors of Trimethoprim and Ultraviolet Light Sensitivities of *Saccharomyces cerevisiae rad6* Mutants†

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Dominant mutations at two newly identified loci, designated *SRS1* and *SRS2*, that metabolically suppress the trimethoprim sensitivity of *rad6* and *rad18* strains, have been isolated from trimethoprim-resistant mutants arising spontaneously in *rad6-1 rad18-2* strains of the yeast *Saccharomyces cerevisiae*. The *SRS2* mutations also efficiently suppress the ultraviolet light sensitivity of the parent strains. They do not, however, suppress their sensitivity to ionizing radiation or their deficiency with respect to induced mutagenesis and sporulation. Such observations support the hypothesis that *RAD6*-dependent activities can be separated into two functionally distinct groups: a group of error-free repair activities that are responsible for a large amount of the radiation resistance of wild-type strains and also for their resistance to trimethoprim, and a group of error-prone activities that are responsible for induced mutagenesis and are also important in sporulation, but which account at best for only a very small amount of wild-type recovery.

The *RAD6* gene of *Saccharomyces cerevisiae* appears to play an essential and central role in a variety of processes that enhance survival and mutagenesis in mutagen-damaged cells, but the number of such processes, their characteristics, and the function of the *RAD6* gene product within them, whether enzymatic or regulatory, are not yet known. Strains carrying the *rad6-1* mutation are more readily killed than wild type by a variety of physical and chemical agents (4, 15), are deficient with respect to induced mutagenesis when treated with such agents (15, 17), show elevated levels of induced and spontaneous recombination (9, 11), exhibit higher rates of spontaneous mutagenesis (7), and are sensitive to growth inhibition by the antifolate drug trimethoprim (5). In addition, diploids homozygous for *rad6-1* and *rad6-3* fail to sporulate (4, 6) and produce no meiotic recombinants, even though they synthesize DNA before meiosis in a normal manner (J. Game, personal communication). Finally, haploid strains carrying *rad6-1* are also unable to repair single- and double-strand breaks induced in their DNA by methyl methane sulfonate (1, 10). Since wild-type haploids repair double-strand breaks only in $G_2$, it is possible that they do so by a recombination-dependent mechanism (10).

The *RAD6*-dependent processes are carried out by a set of at least 9, and probably more than 14, genes that together form a single epistasis group with respect to UV (12) and gamma-ray survival (McKee and Lawrence, unpublished data). Strains that are mutant with respect to other genes within the *RAD6* group never exhibit all of the above phenotypes, however, and the manifestation of any one of these characteristics is rarely as extreme (4, 12, 13). Such observations suggest that there may be a number of functionally distinct processes dependent on *RAD6* gene activity. Moreover, the diversity of these processes raises the possibility that the protein product of the *RAD6* locus is a regulatory, rather than enzymatic, molecule. The existence of a protein product is implied by the finding that both the *rad6-1* and *rad6-3* alleles can be translationally suppressed (unpublished data).

We have investigated the possibility that the *RAD6* gene product is active in a variety of different processes, and we also have attempted to place these in functionally distinct groups, in three separate ways: by a detailed examination of the phenotypes of strains that carry one or more nonallelic mutations in the *RAD6* cluster (12–15; C. W. Lawrence and R. B. Christensen, Genetics, in press), by determining the properties of the new *rad6-4* mutation (J. Douthwright-Fasse, R. B. Christensen, and C. W. Lawrence, unpublished data), and by the isolation of metabolic suppressors of *rad6* mutations, the subject of the present report.

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A possible means of easily selecting such mutations from the mutationally refractory *rad6* strains was suggested to us by the work of Game et al. (5), which showed that the growth of *rad6* and *rad18* strains was inhibited by the antifolate drug trimethoprim, though the growth of wild-type strains or strains mutant at 1 of 26 other *rad* loci was completely unaffected by this agent. These authors also found that *rad6-1 rad18-1* double mutant strains exhibited a high frequency of spontaneous mutation to trimethoprim resistance, giving rise to clones which in some instances possessed a sensitivity to killing by UV light that was intermediate between the parental and wild-type strains (5), a phenotype that might indicate metabolic suppression.

Genetic analysis of 13 independent trimethoprim-resistant derivatives newly isolated from *rad6-1 rad18-2* strains shows that the majority do in fact contain metabolic suppressors. Such suppressor mutations are dominant and identify two new genes, designated *srs1* and *srs2* (for suppressor of rad six). Apart from their resistance to trimethoprim, strains carrying *SRS1* mutations seem to be identical in all respects to the parent strain, a phenotype suggesting that they may be impermeable to the drug. Strains carrying *SRS2* mutations, on the other hand, are also resistant to UV, though not to gamma rays. Furthermore, *SRS2* mutations do not suppress the deficiency of *rad6* mutants with respect to induced mutagenesis or sporulation.

The properties of the strains carrying *SRS2* mutations support the hypothesis that there are at least two types of *RAD6*-dependent activities: nonmutagenic repair of recovery processes that are responsible for a substantial fraction of wild-type resistance to radiations and also for resistance to growth inhibition by trimethoprim, but which are not concerned either with induced mutagenesis or sporulation; and a set of mutagenic processes (12–14; Lawrence and Christensen, Genetics, in press) which contribute little to radiation resistance but are essential for induced mutagenesis and sporulation. This conclusion is also supported by the work of Douthwright-Fasse et al. (manuscript in preparation), which shows that the *rad6-4* mutation has a phenotype that is the opposite of the *rad6-1 SRS2* mutant. Finally, these results suggest that sensitivity to the antifolate drug trimethoprim may be the consequence of the ability of this agent to damage DNA, and, conversely, that resistance to it results from repair of this damage, a conclusion that may be of significance in cancer therapy, where similar drugs are used.

## MATERIALS AND METHODS

**Strains.** Trimethoprim-resistant derivatives were isolated from each of two *rad6-1 rad18-2* double mutant strains, the genotypes of which are given in Table 1. Three of these (F-629, F-641, F-650) were chosen as representative of their phenotypic class (see Table 2) and were crossed with either CL31-2D or CL46-3B to obtain *RAD6*$^+$ segregants that carried *rad18-2* and the corresponding suppressor, for use in subsequent crosses, as well as *rad6-1 rad18-2 SUP* segregants of mating type opposite to that of the parental strain. These strains were used to construct a series of diploids, homozygous for *cyc1-9 rad6-1* and *rad18-2*, that were either homozygous or heterozygous for one or other of the three representative suppressors. Such diploids were studied to determine the dominance of the suppressors and also their effect on the UV-induced reversion of *cyc1-9* and on UV and gamma ray survival. The haploid segregants carrying the suppressors were also crossed with strains multiply marked with amber and ochre auxotrophic mutations and with strains carrying *rad6-3*, *rad18-3*, or *rad18-4*. Finally, some of these haploids were crossed with *rad1-2* strains to study the effect of the suppressor from F-629 (*SRS2-1*) on UV survival in an excision-deficient background. The genotypes of the various strains used in these crosses are given in Table 1.

**Trimethoprim sensitivity.** Sensitivity to trimethoprim was determined in one of two ways: by spreading between $10^2$ and $10^3$ well-washed cells on trimethoprim-supplemented synthetic medium and also on unsupplemented control plates, or by replica-plating onto such media, using a rod-type replicator which transfers drops of cell suspension. With either method it is important to avoid contaminating the cell suspensions with traces of yeast extract-peptone medium, since this diminishes or abolishes the sensitivity of *rad6* and *rad18* strains to trimethoprim. Similarly, it is also important to avoid high cell concentrations, since they have the same effect. In the replicator method, the desired conditions were achieved by serial dilution; samples of the original cell suspensions, made by pick-

TABLE 1. *Genotype and source of strains*

| Strain | Genotype | Source |
|---|---|---|
| CL167-8C | **a** *cyc1-9 rad6-1 rad18-2 leu1-12 arg4-17 his5-2 ade2-1* | This laboratory |
| CL167-11D | α *cyc1-9 rad6-1 rad18-2 met1-1 arg4-17 his5-2 ade2-1* | This laboratory |
| CL31-2D | **a** *rad18-2 lys1-1 ura4-11* | This laboratory |
| CL46-3B | α *rad18-2 lys1-1 lys2-1 trp2* | This laboratory |
| SL210-3A | **a** *met8-1 aro7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1* | S. Liebman |
| SL158-23B | α *met8-1 aro7-1 trp1-1 ade3-26 his5-2 lys1-1* | S. Liebman |
| LP204-1B | **a** *rad6-3 ilv3 lys2-1 his1 trp2* | L. Prakash |
| rs-18 | α *rad18-3* | R. Snow |
| rs-28 | α *rad18-4* | R. Snow |
| CL833-26B | α *cyc1-91 rad1-2 lys2-1 his1-1* | This laboratory |
| CL834-36C | α *cyc1-363 rad1-2 lys2-1 arg4-17* | This laboratory |

ing up cells from a master plate with the replicator and transferring them to wells filled with sterile water, were transferred with the aid of the replicator to a second set of water-filled wells.

**Media.** Synthetic dextrose media contained Difco yeast base (without ammonium sulfate or amino acids), 0.17%; ammonium sulfate, 0.5%; dextrose, 2%; and Difco Noble agar, 1.5%, supplemented with nutrilites or trimethoprim (2,4-diamino-5-[3,4,5-trimethoxy-benzyl]-pyrimidine, 200 mg/liter) where necessary. Yeast extract-peptone-dextrose medium, containing Difco yeast extract, 1%; Difco peptone, 2%; and dextrose, 2% (solidified with Difco agar, 1%, where necessary), was used as standard growth medium. *cyc1* revertants were selected on semisynthetic lactate medium, similar to synthetic dextrose medium but containing yeast extract, 0.05%, and DL-lactate, 1% (vol/vol), instead of dextrose. Comparable estimates of viability were obtained on yeast extract-peptone-glycerol medium, similar to yeast extract-peptone-dextrose medium but containing glycerol, 2% (vol/vol), instead of dextrose.

**Survival curves and mutation induction.** Strains were grown for 2 or 3 days with vigorous shaking at 30°C, washed by centrifugation, and handled in the manner described previously (12–15). Details of the radiation sources and their dosimetry can also be found in earlier publications (12, 15).

**Estimates of spontaneous mutation rates.** About 50 cells of either CL167-8C or CL167-11D were spread on synthetic dextrose medium containing twice the normal amount of agar, and the plates were incubated until each colony contained about $5 \times 10^6$ cells. Cells from a single colony were spread on each of 25 plates containing synthetic dextrose medium supplemented with trimethoprim by cutting out blocks of agar containing a colony and removing most of the cells with a spreader and the remainder by washing with 0.2 ml of sterile water. The number of cells in each of five colonies was estimated by suspending them in 1 ml of water and counting the number of cells with a hemacytometer. After these plates were incubated for 3 days, they were scored for the total number of trimethoprim-resistant colonies, and all colonies were transferred by toothpicks to yeast extract-peptone-dextrose master plates. Replicas of the master plates were exposed to graded series of gamma-ray doses or UV fluences to identify the number of class I, class II, and class III mutants. The total spontaneous mutation rate was estimated by the method of the median (16), and those for individual classes of mutants were estimated by the $P_0$ method (16).

## RESULTS

**Mutant isolation and genetic analysis.** Twenty-eight spontaneous trimethoprim-resistant derivatives were isolated from six independent clones of CL167-8C and six of CL167-11D. The UV and gamma-ray sensitivity of these mutants was examined by exposing replicas of a master plate containing them to a graded series of doses. On the basis of their sensitivity to these radiations, the mutants could be placed in one of three different classes: mutants in class I were as sensitive to both radiations as the parental strains; those in class II were UV resistant but gamma-ray sensitive; and those in class III were resistant to both radiations. Thirteen of these mutants (three from class I, eight from class II, and two from class III; Table 2) were chosen for further study. All possible mutants were chosen, except that only a single representative of any one class was isolated from each clone, therefore ensuring that each spontaneous mutation was of independent origin.

Crosses of each of the 13 mutants to one or the other of the two parental strains, CL167-8C

TABLE 2. *Origin and properties of mutant strains*

| Class | Strain | Parent | Sensitivity[a] | | Sporulation *rad6/rad6* | Dominance | Growth rate[b] | Mutant allele |
|---|---|---|---|---|---|---|---|---|
| | | | UV | Gamma ray | | | | |
| I | F-641 | 8C | S | S | – | + | Equal | *SRS1-1* |
| | F-631 | 8C | S | S | – | + | | *SRS1-2* |
| | F-645 | 11D | S | S | – | + | | *SRS1-3* |
| II | F-629 | 8C | R | S | – | + | Equal | *SRS2-1* |
| | F-554 | 8C | R | S | – | + | | *SRS2-2* |
| | F-555 | 11D | R | S | – | + | | *SRS2-3* |
| | F-630 | 8C | R | S | – | + | | *SRS2-4* |
| | F-638 | 8C | R | S | – | + | | *SRS2-5* |
| | F-642 | 8C | R | S | – | + | | *SRS2-6* |
| | F-643 | 11D | R | S | – | + | | *SRS2-7* |
| | F-648 | 11D | R | S | – | + | | *SRS2-8* |
| III | F-650 | 11D | R | R | + | + | Faster | *SUP* amber |
| | F-652 | 11D | R | R | ± | + | | *SUP* omni |

[a] S, Sensitive (= to parental strain); R, resistant. See Fig. 1 and 2.
[b] Growth rate relative to that of parent strain; see Table 7.

or CL167-11D, showed that the mutations they carried were dominant with respect to trimethoprim resistance and, in the class II or class III mutants, also with respect to radiation resistance. The mutations in class I and class II mutants did not suppress the sporulation defect of these *rad6-1* homozygous diploids, although the mutation in F-650, a class III mutant, did so efficiently. The diploid obtained by crossing F-652 with CL167-8C also sporulated, but only to a very small extent.

The mutant strains F-641, F-629, and F-650 were chosen as representative of mutants in class I, class II, and class III, respectively. Each was crossed with either CL46-3B or CL31-2D, to isolate the mutations in these strains in a *rad18* background free of *rad6-1*, and also with wild-type strains. Sporulation of the diploids homozygous for *rad18-2*, followed by analysis of haploid segregants, showed that the trimethoprim resistance of each of the three strains, F-641, F-629, and F-650, was the consequence of a single mutation; trimethoprim sensitivity segregated 2:2 in each set of five tetrads tested. Furthermore, UV resistance cosegregated with trimethoprim resistance in the crosses involving F-629 and F-650, showing that the same mutation was responsible for these two properties. Finally, these crosses, and also those with wild-type strains, show that the mutations in F-641, F-629, and F-650 are all unlinked to either the *rad6* or the *rad18* locus.

Since the mutations in each of the 13 strains analyzed are dominant, it is not possible to determine whether all of the members of a given class contain alleles of a single gene locus by the criterion of the inability of these mutations to complement one another. Instead, strains carrying the mutations derived from the three representative mutants were crossed with all other members of the same phenotypic class, and also with the representative strains from the other two classes. Each of these diploids was sporulated, from five to nine tetrads were dissected, and the trimethoprim sensitivity of the segregants was determined (Table 3). All crosses between strains carrying mutations from the same phenotypic class gave parental ditype tetrads exclusively, in which all four spore clones were resistant to trimethoprim, with the exception of the cross between the two class III mutants, which did not sporulate and therefore could not be tested. Crosses between strains bearing mutations from mutants of different class gave tetratype tetrads predominantly, in which one of the spore clones was sensitive to trimethoprim and the other three were resistant. These results indicate that all of the mutations in class I are probably alleles of a single gene, which we designate *SRS1* (for suppressor of rad six), and that all class II mutations are probably alleles of a different gene, *SRS2*, unlinked to *SRS1*. Phenotypic evidence, discussed below, suggests that the two mutations in class III are located at different loci active in translation.

TABLE 3. *Linkage analysis of trimethoprim resistance mutations*

| Class | Mutants crossed | | Segregation, trim$^r$: trim$^s$[a] | | | Total no. of tetrads |
|---|---|---|---|---|---|---|
| | Class | Source | 4:0 (PD) | 3:1 (T) | 2:2 (NPD) | |
| I × I | F-641 | F-631 | 5 | 0 | 0 | 5 |
| | F-641 | F-645 | 6 | 0 | 0 | 6 |
| II × II | F-629 | F-554 | 5 | 0 | 0 | 5 |
| | F-629 | F-555 | 6 | 0 | 0 | 6 |
| | F-629 | F-630 | 6 | 0 | 0 | 6 |
| | F-629 | F-638 | 5 | 0 | 0 | 5 |
| | F-629 | F-642 | 6 | 0 | 0 | 6 |
| | F-629 | F-643 | 9 | 0 | 0 | 9 |
| | F-629 | F-648 | 5 | 0 | 0 | 5 |
| I × II | F-641 | F-629 | 1 | 3 | 1 | 5 |
| I × III | F-641 | F-650 | 0 | 4 | 1 | 5 |
| II × III | F-629 | F-650 | 0 | 5 | 0 | 5 |

[a] trim$^r$, trim$^s$, Resistance and sensitivity to trimethoprim, respectively; PD, parental ditype; T, tetratype; NPD, nonparental ditype.

**Effect of suppressors on other alleles.** Strains carrying *SRS1-1*, *SRS2-1*, *SRS2-2*, *SRS2-3*, and the suppressors present in F-650 and F-652 were crossed with SL158-23B, or SL210-3A and a number of other strains; the diploids were sporulated, and from 6 to 16 tetrads were dissected to examine the effect of the suppressors on the amber and ochre suppressible alleles that these strains contain. These crosses (Table 4) provided no evidence to indicate that the *SRS-1* or any of the *SRS2* suppressors were capable of nonsense suppression; segregants carrying the suppressor and an amber or ochre auxotrophic mutation showed no growth on minimal medium even after 7 days of incubation at 30°C (Table 4). In contrast, the suppressors present in F-650 and F-652 both showed evidence of nonsense suppression after incubation for 1 day; the F-650 suppressor acted only on amber alleles and is therefore presumably an amber suppressor of the tRNA variety, whereas the F-652 suppressor acted on both amber and ochre alleles and is therefore presumably an omnipotent suppressor (8), a mutation in a gene coding for a ribosomal component (J. Wilhelm, personal communication). Although both *rad6-1* and *rad18-2* are amber suppressible alleles, *rad18-2* is not suppressed by tyrosyl tRNA suppressors, the most common class of amber sup-

TABLE 4. *Effect of the suppressors on nonsense alleles*

| Suppressor | Suppressible alleles[a] | | | | | | | | | | No. of tetrads tested | Type of suppressor[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amber alleles | | | | | Ochre alleles | | | | | | |
| | *ade3-26* | *aro7-1* | *ilv1-1* | *met8-1* | *trp1-1* | *ade2-1* | *arg4-17* | *his5-2* | *leu2-1* | *lys1-1* | | |
| *SRS1-1* | – | – | NT | – | – | NT | – | – | NT | – | 12 | M |
| *SRS2-1* | – | – | – | – | – | NT | – | – | – | – | 6 | M |
| *SRS2-2* | NT | – | NT | NT | – | – | – | – | NT | – | 9 | M |
| *SRS2-3* | NT | – | NT | NT | – | – | – | – | – | – | 12 | M |
| *SUP* F-650 | + | + | NT | + | + | NT | – | – | NT | – | 16 | TA |
| *SUP* F-652 | NT | + | + | NT | + | NT | + | – | + | + | 7 | TO |

[a] +, Suppression; –, no suppression; NT, not tested.
[b] M, Metabolic; TA, translational, amber; TO, translational, omnipotent.

pressors, and this no doubt accounts for the relatively low frequency of translational suppressors among the trimethoprim-resistant strains.

These results suggest that *SRS1* and *SRS2* are nontranslational, or metabolic, suppressors of *rad6* and *rad18* mutations. To substantiate this point, the ability of the *SRS2-2* and *SRS2-3* mutations to suppress *rad6* and *rad18* alleles other than *rad6-1* and *rad18-2* was determined. To this end, strains carrying these suppressors and either *rad6-1* or *rad18-2* were crossed either with strains carrying *rad18-3* and *rad18-4* or with *rad6-3*, respectively; the diploids were sporulated, and eight tetrads were dissected. Each of the 32 segregants was crossed with *rad6-1* and *rad18-2* tester strains to identify the *rad* alleles they carry by complementation, and each was also tested for trimethoprim sensitivity, using a whole plate test for each segregant. These tests showed that the *SRS2-3* and, in those cases examined, the *SRS2-2* mutation suppressed the trimethoprim sensitivity associated with all *rad6* and *rad18* alleles. *SRS2-1* has also been shown to suppress the trimethoprim and UV sensitivity due to a third *rad6* allele, *rad6-4* (Douthwright-Fasse et al., manuscript in preparation). The suppressor activity of *SRS2* mutations is therefore locus specific–allele nonspecific and suppresses only some of the phenotypes of *rad6* mutants, indicating that the mutations are nontranslational or metabolic, rather than translational, suppressors of *rad6*.

**Survival and induced mutagenesis.** UV survival curves of the 13 trimethoprim-resistant derivatives, together with those of the two parental strains (Fig. 1), confirm the qualitative results of the spot tests referred to above and also demonstrate quantitatively the extent of the suppression of UV sensitivity by class II and class III suppressors. The class III translational suppressors (in F-650 and F-652) are the most effective in this respect, whereas the *SRS2* (class II) mutations suppress *rad6-1* and *rad18-2* UV sensitivity to varying extents; the *SRS2-1* mutation in F-629 is almost as efficient as the amber suppressor in F-650, and the *SRS2-3* mutation in F-555 is much less effective. The *SRS1* strains (F-631, F-641, F-645) are all as UV sensitive as the two parental strains.

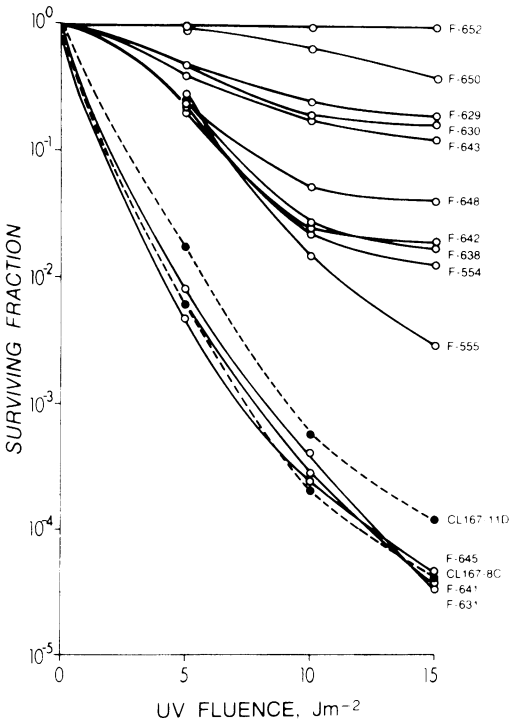


FIG. 1. *UV survival curves for the 2 parental strains CL167-8C and CL167-11D (broken lines) and the 13 trimethoprim-resistant derivatives (unbroken lines). F-631, F-641, and F-645 are class I mutants (SRS1 alleles), F-650 and F-652 are class III mutants (translational suppressors), and the remainder are class II mutants (SRS2 alleles).*

Although the *SRS2* mutations can suppress

the UV sensitivity of the *rad6-1 rad18-2* parental strain with high efficiency, they nevertheless have no effect at all on their gamma-ray sensitivity (Fig. 2). Similarly, the *SRS1* suppressors also have no influence on gamma-ray sensitivity, and only the two translational suppressors have any effect in this respect.

The *SRS2-3* mutation suppresses UV sensitivity not only of *rad6-1 rad18-2* double mutants, but also of *rad6-1* and *rad18-2* single mutant strains (Fig. 3). The results in Fig. 3 are the average of data from two segregants taken from a cross between F-555 and a wild-type strain. All segregants were crossed to *rad6-1* and *rad18-2* tester strains, to determine their *rad* genotype by complementation, and were tested for trimethoprim sensitivity. The $RAD^+$ segregants were taken from tetrads in which it was possible to infer the presence or absence of the *SRS2* mutation, assuming a 2:2 segregation for this allele, since this mutation has no detectable phenotype in $RAD^+$ strains. The effect of *SRS2* mutations on the survival of $RAD^+$ strains is at best very small, and probably nonexistent, even at a fluence of 150 $J \cdot m^{-2}$, where only about 1% cells can form colonies (data not shown).

The results in Fig. 4 and 5 demonstrate that

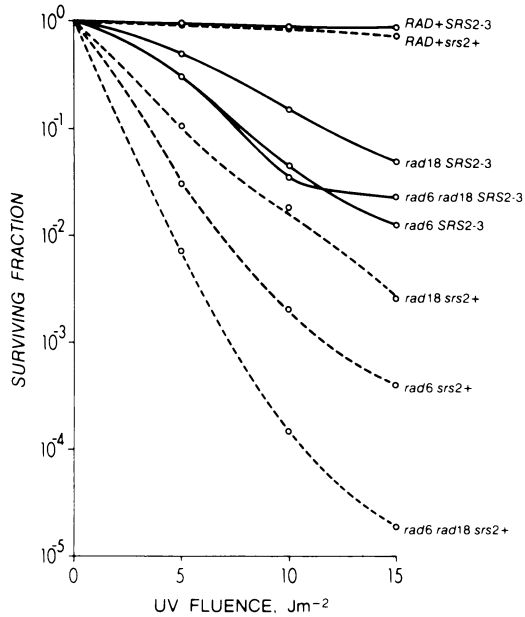


FIG. 3. *UV survival curves for haploid strains carrying all combinations of rad6-1, rad18-2, SRS2-3, or their wild-type alleles. Data are the averages from two replicate strains.*

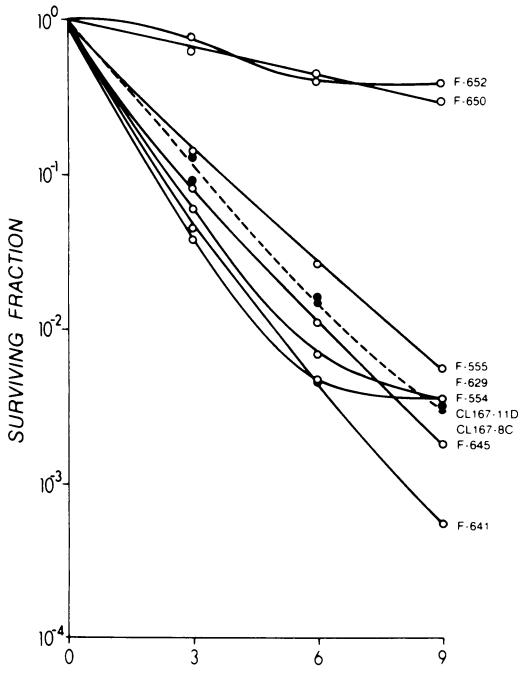


FIG. 2. *$^{60}$Co gamma-ray survival curves for the two parental strains CL167-8C and CL167-11D (broken lines) and representative examples of class I, II, and III mutants (unbroken lines).*

the class II and class III suppressors are dominant with respect to UV survival and that the class III amber suppressor is similarly dominant for gamma-ray survival, and therefore confirm the conclusion reached from the much less reliable spot tests referred to above. Although the survival curves in Fig. 4 appear to indicate that the *SRS2-1* mutation is not fully dominant, the variability between replicate determinations was large enough to make this conclusion doubtful. The results also show that the suppressors have a qualitatively and quantitatively similar effect in diploid and haploid strains. The presence of a large tail to the gamma-ray survival curve for the *SRS2-1* heterozygote is the consequence of cell aggregates that could not be dispersed by sonication; it is unlikely that the inherent resistance of this strain to gamma rays is materially different from that of the *SRS2-1* homozygote.

Although the *SRS2* mutation suppresses the UV sensitivity of *rad6* strains, it does not, however, suppress their deficiency with respect to UV-induced mutagenesis. Table 5 shows the frequency of revertants of the ochre allele *cyc1-9* induced by a 5-$J \cdot m^{-2}$ UV dose in diploids homozygous for *rad6-1* and *rad18-2* and heterozygous for *SRS1-1*, *SRS2-1*, or the amber suppressor, as well as an unsuppressed *rad6-1 rad18-2* strain and a $RAD^+$ control. These results show that the *SRS2-1* and also the *SRS1-*

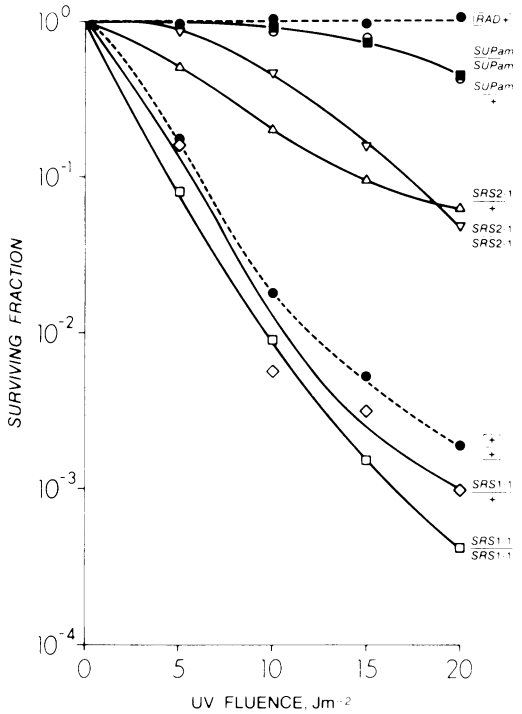


FIG. 4. *UV survival curves for diploid strains homozygous for rad6-1 and rad18-2 and also homozygous or heterozygous for SRS1-1, SRS2-1, or the amber suppressor from F-650. The strain marked + / + carries no suppressor, whereas that marked RAD⁺ is heterozygous for rad6-1 and rad18-2 (broken lines). Data are the averages of two experiments.*

*1* heterozygote is as immutable by UV as the *rad6-1 rad18-2* control diploid. Similar results were obtained in haploid strains in which the UV-induced reversion of *arg4-17* was examined (data not shown). As might be expected, translational suppression of *rad6-1* restored the capacity for UV mutagenesis, though the extent of this restoration was much less than it was for survival.

**Effect of *SRS2* in excision-defective strains.** Since *SRS2* mutations suppress the UV sensitivity but not the mutational deficiency of *rad6* strains, such mutations presumably restore to these strains a capability for nonmutagenic or error-free repair or recovery. Moreover, the efficiency of the suppression implies that this nonmutagenic repair has the capacity for coping with a large amount of damage, comparable to that normally handled by *RAD6*-dependent processes or by excision. *SRS2* repair is not, however, the consequence of an enhancement of excision repair, present to a normal extent in unsuppressed *rad6-1* strains (18), nor yet does it appear to make use of a normal excision process, since the *SRS2-1* mutation efficiently suppresses

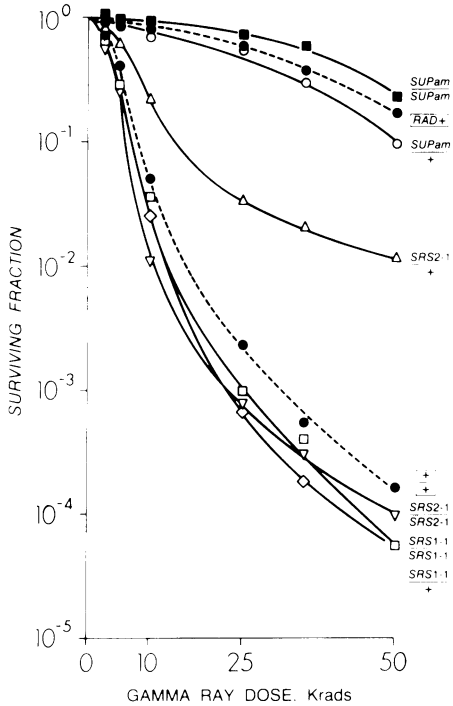


FIG. 5. *$^{60}$Co gamma-ray survival curves for the same diploid strains listed in Fig. 4. Data are the averages of two experiments.*

TABLE 5. *UV-induced reversion of cyc1-9 in rad6-1 rad18-2 diploid strains heterozygous for one of the three representative suppressors and in an RAD⁺ control*

| Strain | Genotype | | | Revertants per $10^8$ survivors (% survival) *cyc1-9 → CYC1⁺* | |
|---|---|---|---|---|---|
| | *rad6* | *rad18* | *sup* | 0 J·m$^{-2}$ | 5 J·m$^{-2}$ |
| CL872 | +/− | +/− | +/+ | 8 (100) | 64 (103) |
| CL849 | −/− | −/− | +/+ | 1 (100) | 1 (44) |
| CL853 | −/− | −/− | *SRS1-1*/+ | 3 (100) | 2 (29) |
| CL848 | −/− | −/− | *SRS2-1*/+ | 0 (100) | 0 (85) |
| CL855 | −/− | −/− | *SUP* amber/+ | 1 (100) | 10 (105) |

the UV sensitivity due to *rad6-1* in excision-deficient strains (Fig. 6). Each of the three survival curves in Fig. 6 is the average of data from four replicate strains.

It is clear from Fig. 6 that the *SRS2-1* suppressor is at least as efficient in excision-deficient strains as it is in excision-proficient haploids, but the extreme sensitivity of *rad1-2 rad6-1 srs2*$^+$ strains and the absence of data points for fluences below 0.25 J·m$^{-2}$ preclude a more exact comparison. The resistant subpopulation in the *rad1-2 rad6-1 srs2*$^+$ haploids, amounting to about 1% of the total cells, has a survival curve very similar to that of the translationally suppressed strains and probably represents spontaneous translational suppressor mutations. The spontaneous mutation rate for these genes is very high, and such mutations accumulate very rapidly in *rad1 rad6* strains (see Table 6). Their presence in all *rad1 rad6* double mutant cell populations makes it impossible to obtain true estimates of double mutant sensitivity, and it is probable that all previous estimates (3, 15) are incorrect. It is possible that the true sensitivity of *rad1 rad6* double mutants is high enough to indicate that a single dimer represents one lethal hit, implying that all repair capacity has been lost in these strains.

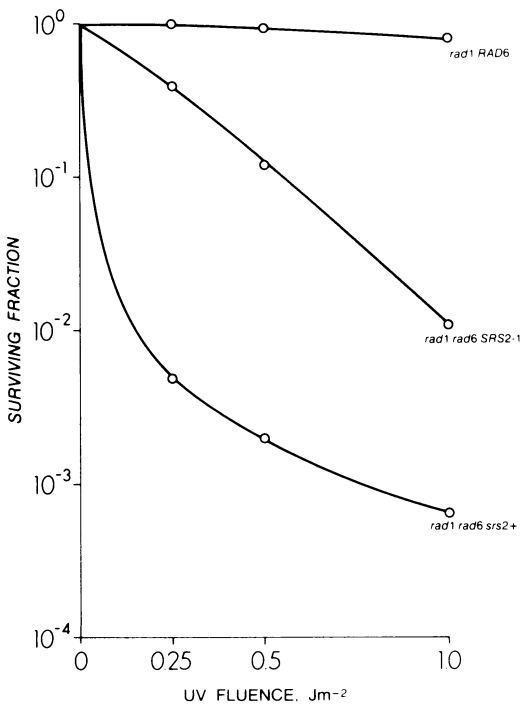


FIG. 6. *UV survival curves for excision-defective haploid strains (carrying rad1-2) that also carry RAD6*$^+$*, rad6-1 SRS2-1, or rad6-1 srs2*$^+$*. Data are the averages from four replicate strains.*

**Spontaneous mutation rates.** As noted above, it is impossible to grow *rad6* or *rad18* strains without accumulating sizable subpopulations of trimethoprim-resistant cells; cultures that have been mass-transferred several times without subcloning may have more than 10% of such cells. This is, no doubt, in part the consequence of the enhanced growth rate of some suppressed mutants, but the data in Table 6 suggest that the rates of spontaneous mutation to trimethoprim resistance are also high; in both parental *rad6 rad18* strains the rate is about seven mutations per $10^7$ cells per division. The individual mutation rates for class III suppressors, obtained by determining the UV and gamma-ray sensitivity of each spontaneous mutant, are probably somewhat inflated due to their selective advantage during the growth of the clone, but this is not the case with class I or class II events. Mutations of these two kinds do not appear to suppress deficiencies in *rad6 rad18* mutants which cause populations of these cells to have such long doubling times (Table 7). It is

TABLE 6. *Spontaneous rates for mutation to trimethoprim resistance in the two parental strains*

| Strain | No. of cultures tested | Mutation rate[a] | | | |
|---|---|---|---|---|---|
| | | Total* | Class I† | Class II† | Class III† |
| CL167-8C | 25 | 6.5 | 3.4 | 3.0 | 1.8 |
| CL167-11D | 25 | 6.7 | 0.8 | 3.4 | 1.7 |

[a] Mutations per $10^7$ cells per division; *, by method of median (16); †, by $P_0$ method (16). The values for the three classes do not add up to the value for the total frequency because they were estimated by different methods.

TABLE 7. *Cell population doubling times for the two parental strains and for two representative strains from each of the three classes of mutants, growing at 30°C in yeast extract-peptone-dextrose medium*

| Strain | Class | Genotype | Doubling time (h) |
|---|---|---|---|
| CL167-8C | Parent | + | 2.7 |
| CL167-11D | | + | 2.4 |
| F-641 | I | *SRS1-1* | 2.6 |
| F-631 | | *SRS1-2* | 2.7 |
| F-629 | II | *SRS2-1* | 2.4 |
| F-554 | | *SRS2-2* | 2.5 |
| F-650 | III | *SUP* amber | 1.5 |
| F-652 | | *SUP* omni | 2.4 |

not known whether these long doubling times reflect an inherently slow growth rate, the poor viability of logarithmically growing cells, or both. The difference between the two parental strains with respect to class I mutation rate may not be real, because of the difficulty of discriminating between class I mutants and very weak class II suppressors. Spontaneous mutation rates for single base substitutions in wild-type cells are about 5 mutations per $10^9$ cells per division (20), which is about 100 times lower than the values given in Table 6, so that either there are many genetic sites within each gene at which these dominant mutations can occur or their mutation frequency is atypically high. Strains carrying either *rad6-1* or *rad18-2* appear to have somewhat higher rates of spontaneous mutation (7, 20), and so the double mutant might be expected to be more extreme in this respect.

## DISCUSSION

The observation that the *SRS2* mutations are capable of suppressing some, but not all, of the *rad6* mutant phenotypes lends support to the hypothesis that *RAD6*-dependent activities are divided into a number of functionally distinct groups. This conclusion is also supported by an examination of the phenotypes of strains carrying other mutations within the *rad6* epistasis group and by the properties of the *rad6-4* mutation (Douthwright-Fasse et al., manuscript in preparation). These pieces of evidence suggest that the *RAD6* cluster of activities is divided into two main groups: a group of at least two error-free repair activities that are responsible for a large part of the radiation resistance of wild-type strains, and also their resistance to trimethoprim; and a group of error-prone activities responsible for the production of mutations of different kinds, at different sites (12–15; Lawrence and Christensen, Genetics, in press), or with different mutagens (17), but which account at best for only a very small amount of wild-type resistance. One or more activities in this second group also appear to play an important part in sporulation (Douthwright-Fasse et al., manuscript in preparation). Acting in concert, the *RAD6* cluster of activities provides yeast cells with the capacity to cope with a highly significant amount of damage inflicted by a variety of DNA-damaging agents (4, 15), for the most part by mechanisms that do not rely either on excision or recombination. Using the classification proposed by Clark and Volkert (2), it is therefore tempting to speculate that the *RAD6* cluster is concerned with intrareplicational repair that is directed towards the restoration of normal DNA synthesis in irradiated cells, but there is no direct evidence to support this view.

The *SRS2* mutations seem to suppress the UV sensitivity of *rad6* strains either by restoring a substantial fraction of the normal *RAD6*-dependent error-free repair to these strains, or possibly by providing an alternative activity capable of carrying out this function. They do not suppress either the mutational or sporulation deficiency of *rad6* mutants, however, suggesting that these are enzymatically different processes. The *SRS2* mutations also suppress the UV sensitivity of *rad18* mutants, and it is significant that these mutants are almost as UV sensitive as *rad6* strains. Like *rad6* strains, they are also sensitive to gamma rays (15, 19), though not to anywhere near the same extent, to chemical mutagens (17), and to trimethoprim, but they differ from *rad6* strains in being normal with respect to sporulation and relatively normal for induced mutagenesis. This phenotype suggests that the *RAD18* gene product is involved in *RAD6*-dependent error-free, but not error-prone, repair processes. Previous data (15) indicating that *rad18-2* strains were deficient with regard to induced mutagenesis have not proved to be typical, and more recent results (unpublished data) show that these strains are much more nearly normal in this respect.

Even though *rad6* and *rad18* mutants share in common a sensitivity to both radiations, the *SRS2* alleles suppress only their UV sensitivity, and not their sensitivity to gamma rays; this observation implies that different processes are responsible for the error-free repair of UV and gamma-ray damage. This conclusion is supported by the properties of *rad9* and *rad15* mutant strains. Both strains are extremely sensitive to gamma rays, *rad9* mutants being as sensitive in this respect as those carrying *rad6* (McKee and Lawrence, manuscript in preparation), both are only moderately sensitive to UV (4, 15) but neither is sensitive to trimethoprim (5), and both are sporulation proficient (6). Viewed as a whole, these observations suggest that there are two main error-free repair processes dependent on *RAD6* gene function: a *RAD18*-dependent process that is concerned predominantly with the repair of UV damage and is also responsible for trimethoprim resistance, and a *RAD9 RAD15*-dependent process that is concerned predominantly with the repair of gamma-ray damage. Since *rad6* mutants are sensitive to a very broad range of DNA-damaging agents, it is possible that there are several more independent error-free repair pathways within this cluster. Apart from this group of at least two error-free repair processes, there is also evidence for several partially independent error-prone processes within the *RAD6* cluster which are responsible for induced mutagenesis (12–15),

as well as an activity, possibly identical with one of these processes, which is responsible for some step in sporulation, perhaps recombination. The *RAD6* group of activities cannot properly be called a "pathway," therefore; it appears to be more exact to regard them as a set of functionally distinct processes, possibly directed towards a common goal such as the restoration of DNA synthesis, that are coordinately regulated by the *RAD6* gene product or are dependent on this product in some other way.

Although it is clear that *SRS2* mutations provide an activity that compensates efficiently for the absence of error-free repair of UV damage in *rad6-1* and *rad18-2* strains, it is not known whether this is the *RAD6*-dependent activity normally present in wild-type cells or an alternative activity capable of carrying out this function. The observations that the *SRS2* mutations can suppress the UV sensitivity of *rad6* and *rad18* mutant strains with high efficiency, act only on certain loci, and have little effect in wild-type strains, tend to favor the former possibility, however. According to this proposal, *srs2*$^+$ could be a gene normally under positive regulation by the *RAD6* locus, with the *RAD6* gene product acting as the antirepressor for this locus and the dominant *SRS2* alleles being operator mutations which prevent efficient binding of repressor. If this is so, it should be possible to isolate *srs2*-deficient mutations that are recessive, have a phenotype of UV but not gamma-ray sensitivity, and interact epistatically with *rad6* mutations. Alternatively, *srs2* might be the structural gene for an enzyme not normally involved in *RAD6*-dependent processes, but which possesses an activity similar to that required for UV repair. In this case, the *SRS2* mutations might be overproducers, giving rise to such high levels of enzyme that it now can compensate for the *rad6* deficiency. Whatever this novel process might be, it cannot be an enhancement of excision repair, nor yet can it depend on excision, since the *SRS2* suppressor acts efficiently in excision-deficient as well as in excision-proficient strains.

The ability of the *SRS2* mutations to simultaneously suppress sensitivity to trimethoprim and UV strongly suggests that the sensitivity of *rad6* strains to this antifolate drug depends not so much on the ability of the drug to halt DNA synthesis as on the inability of *rad6* mutants to repair the DNA damage, possibly in the form of aberrant replication forks, that this inhibition produces. If so, wild-type cells may be resistant to trimethoprim by virtue of their repair capacity, whereas *SRS1* mutations have the appearance of being impermeable to the drug, though this has not been tested. The possibility that resistance to antifolate drugs depends on DNA repair may have important implications for cancer therapy, in which drugs of this type are used.

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## LITERATURE CITED

1. **Chlebowicz, E., and W. J. Jachymczyk.** 1979. Repair of MMS-induced DNA double-strand breaks in haploid cells of *Saccharomyces cerevisiae*, which requires the presence of a duplicate genome. Mol. Gen. Genet. **167:** 279–286.
2. **Clark, A. J., and M. R. Volkert.** 1978. A new classification of pathways repairing pyrimidine dimer damage in DNA, p. 57–72. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA repair mechanisms. Academic Press Inc., New York.
3. **Cox, B. S., and J. C. Game.** 1974. Repair systems in *Saccharomyces cerevisiae*. Mutat. Res. **26:**257–264.
4. **Cox, B. S., and J. M. Parry.** 1968. The isolation, genetics and survival characteristics of ultraviolet light-sensitive mutants in yeast. Mutat. Res. **6:**37–55.
5. **Game, J. C., J. G. Little, and R. H. Haynes.** 1975. Yeast mutants sensitive to trimethoprim. Mutat. Res. **28:**175–182.
6. **Game, J. C., and R. K. Mortimer.** 1974. A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. **24:** 281–292.
7. **Hastings, P. J., S.-K. Quah, and R. C. vonBorstel.** 1976. Spontaneous mutation by mutagenic repair of spontaneous lesions in DNA. Nature (London) **264:** 719–722.
8. **Hawthorne, D. C., and V. Leupold.** 1974. Suppressors in yeast. Curr. Top. Microbiol. Immunol. **64:**1–47.
9. **Hunnable, E. G., and B. S. Cox.** 1971. The genetic control of dark recombination in yeast. Mutat. Res. **13:** 297–309.
10. **Jachymczyk, W. J., E. Chelbowicz, Z. Swietlinska, and J. Zuk.** 1977. Alkaline sucrose sedimentation studies of MMS-induced DNA single-strand breakage and rejoining in the wild type and in UV-sensitive mutants of *Saccharomyces cerevisiae*. Mutat. Res. **43:**1–10.
11. **Kern, R., and F. K. Zimmermann.** 1978. The influence of defects in excision and error-prone repair on spontaneous and induced mitotic recombination and mutation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **161:**81–88.
12. **Lawrence, C. W., and R. B. Christensen.** 1976. UV mutagenesis in radiation-sensitive strains of yeast. Genetics **82:**207–232.
13. **Lawrence, C. W., and R. B. Christensen.** 1978. Ultraviolet-induced reversion of *cyc1* alleles in radiation-sensitive strains of yeast. I. *rev1* mutant strains. J. Mol. Biol. **122:**1–21.
14. **Lawrence, C. W., and R. B. Christensen.** 1978. Ultraviolet-induced reversion of *cyc1* alleles in radiation-sensitive strains of yeast. II. *rev2* mutant strains. Genetics **90:**213–226.
15. **Lawrence, C. W., J. W. Stewart, F. Sherman, and R. Christensen.** 1974. Specificity and frequency of ultraviolet-induced reversion on an iso-1-cytochrome *c* ochre mutant in radiation-sensitive strains of yeast. J. Mol. Biol. **85:**137–162.
16. **Lea, D. E., and C. A. Coulson.** 1949. The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:**264–285.

17. **Prakash, L.** 1974. Lack of chemically induced mutation in repair-deficient mutants of yeast. Genetics **78:**1101–1118.

18. **Prakash, L.** 1977. Repair of pyrimidine dimers in radiation-sensitive mutants *rad3*, *rad4*, *rad6* and *rad9* of *Saccharomyces cerevisiae*. Mutat. Res. **45:**13–20.

19. **Resnick, M. A.** 1969. Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. Genetics **62:**519–531.

20. **vonBorstel, R. C., K. T. Cain, and C. M. Steinberg.** 1971. Inheritance of spontaneous mutability in yeast. Genetics **69:**17–27.