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RAD6$^+$ Gene of *Saccharomyces cerevisiae* Codes for Two Mutationally Separable Deoxyribonucleic Acid Repair Functions

MICK F. TUITE[1]* AND BRIAN S. COX[2]

Department of Biological Chemistry, California College of Medicine, University of California, Irvine, California 92717,[1] and Botany School, University of Oxford, Oxford, OX1 3RA, England[2]

The response of two mutant alleles of the *RAD6*$^+$ gene of *Saccharomyces cerevisiae* to the ochre translational suppressor *SUQ5* was determined. Both the ultraviolet sensitivity phenotype and the deficiency in ultraviolet-induced mutagenesis phenotype of the *rad6-1* allele were suppressed in a [*psi*$^+$] background. For the *rad6-3* allele, only the ultraviolet-sensitivity phenotype was suppressible in a [*psi*$^+$] background. An *SUQ5 rad6-3* [*psi*$^+$] strain that was examined showed the normal *rad6-3* deficiency in ultraviolet-induced mutagenesis. We propose that the *RAD6*$^+$ gene is divided into two cistrons, *RAD6A* and *RAD6B*. *RAD6A* codes for an activity responsible for the error-prone repair of ultraviolet-induced lesions in deoxyribonucleic acid but is not involved in a cell's resistance to the lethal effects of ultraviolet light. *RAD6B* codes for an activity essential for error-free repair of potentially lethal mutagenic damage.

Genetic evidence has suggested that the yeast *Saccharomyces cerevisiae* has both error-free and error-prone modes of repair of ultraviolet (UV)-induced damage. The major error-free pathway, controlled by the *RAD3*$^+$ gene, is involved in the excision of pyrimidine dimers. A second, minor error-free pathway, controlled by the *RAD51*$^+$ gene, and a single error-prone pathway, controlled by the *RAD6*$^+$ gene, exist. Mutagenesis in *S. cerevisiae*, by both chemical and physical agents, requires a functional *RAD6*$^+$ gene (12, 17). Recent evidence has suggested that the *RAD6* gene may be a coordinate regulator of two independent repair pathways, one being error-free repair, the other error-prone repair (13-15).

In this communication we present evidence that the *RAD6* gene codes for two mutationally separable functions. These control the amounts of error-free repair and mutagenesis observed in UV-irradiated cells. Lawrence and Christensen (13) have described metabolic suppressors of *rad6* mutations that overcome the UV sensitivity of these mutants but not their lack of mutability. We found that, in contrast, informational (translational) suppressors may either do this or restore mutability without significantly affecting survival, depending on the *rad6* allele involved.

MATERIALS AND METHODS

Strains. The haploid yeast strains used in this study are described in Table 1. These strains were constructed by standard tetrad analysis, and the genotypes of haploid segregants were determined as previously described (B. S. Cox, M. F. Tuite, and C. J. Mundy, Genetics, in press). In *RAD*$^+$ × *rad6* crosses, UV-sensitive segregants were detected by a drop test. A 10-μl portion of a saline suspension of each segregant to be tested was spotted onto YC plates (see below) and allowed to dry. The plates were then irradiated at either 20 J/m^2 or 60 J/m^2 doses of UV. Growth or nongrowth was scored after 3 days of incubation in the dark at 28°C.

Media and growth conditions. Strains were grown for 2 to 3 days on YC agar plates at 28°C. YC is a rich medium that has been previously described (4).

UV mutagenesis. For determining UV survival curves and frequencies of UV-induced mutation, cells were resuspended in saline (ca. 5 × 10^6 cells per ml). Conditions for exposure to UV were as previously described (M. F. Tuite and B. S. Cox, Genetics, in press). The isolation of nonsuppressed mutants and determination of their genotypes were done as previously described (Cox et al., in press).

RESULTS

Suppression of *rad6-1*. The *rad6-1* strain 197/2d, originally isolated by Cox and Parry (5), is both highly UV and X-ray sensitive and is blocked in mutation induction by both these and chemical mutagens (12, 17). As part of a study of the effects of radiation-sensitive, deoxyribonucleic acid-repair mutants on the induction of cytoplasmic mutations by UV (Tuite and Cox, in press) we constructed the strain MT201/2c (Table 1) carrying the *rad6-1* mutation together with the serine-inserting ochre suppressor *SUQ5*. UV survival data for this strain showed that it was markedly more resistant to the lethal

TABLE 1. *Genotypes and sources of strains used*

Strain	Genotype	Source
197/2d	α *ade2-1 rad6-1 [psi⁻]*	This lab
466/4a	α *SUQ5 ade2-1 his5-2 can1-100 [psi⁺]*	This lab
465/2a	**a** *ade2-1 his5-2 can1-100 ura3-1 [psi⁺]*	This lab
MT201/2c	α *SUQ5 ade2-1 rad6-1 [psi⁺]*	197/2d × 466/4a
MT209/7b	α *SUQ5 ade2-1 his5-2 rad6-1 [psi⁺]*	MT201/2c × 465/2a
507-4/2b	α *SUQ5 ade2-1 his5-2 can1-100 ura3-1 [psi⁺]*	This lab
JF9-4D	**a** *rad6-3 lys1-1 his1-1 arg4-17 trp2*[a]	C. Lawrence
MT221/1b	α *SUQ5 ade2-1 his1-1 ura3-1 rad6-3 [psi⁺]*	JF9-4D × 507-4/2b

[a] [psi] phenotype not determined.

effects of UV than its *rad6-1* parent 197/2d (Fig. 1), but slightly more UV sensitive than its *RAD*⁺ parent 466/4a. A second strain with the same genotype as MT201/2c (MT209/7b) was therefore constructed (Table 1). It too had a marked increase in its UV resistance, compared with 197/2d (Fig. 1). In both sets of crosses, in which both *SUQ5* and *rad6-1* were segregating, only *sup*⁺ *rad6-1* segregants were detectably UV sensitive by the drop test.

A [*psi*⁻] mutant of MT209/7b was obtained by dimethyl sulfoxide mutagenesis (M. F. Tuite, B. S. Cox, and C. J. Mundy, submitted for publication), and this strain showed an increase in its UV sensitivity, compared with MT209/7b [*psi*⁺](Fig. 1). The suppressor *SUQ5* is more efficient in a [*psi*⁺] genetic background (3). These results strongly suggest that the *rad6-1* mutation is an ochre-suppressible allele of *rad6*.

We next investigated the ability of *SUQ5* to suppress a second *rad6-1* phenotype, namely its failure to show UV-induced mutagenesis. Mutants giving loss of suppression in strains of the genotype *SUQ5 ade2-1* [*psi*⁺] are easily scored as red colonies on YC plates (Cox et al., in press). Loss of suppression of the *ade2-1* ochre mutation allows cells to accumulate the red pigment characteristic of *ade2* mutants. Several different classes of mutation give rise to a nonsuppressed phenotype. These include second-site mutations within the *SUQ5* locus (*suq5*⁺), dominant and recessive antisuppressors, mutations within genes required for the maintenance of the [*psi*⁺] phenotype (*PNM*), and mutations from [*psi*⁺] to [*psi*⁻] (Cox et al., in press).

The *SUQ5 ade2-1 rad6-1* [*psi*⁺] strain MT209/7b showed, per unit dose of UV, a slightly higher frequency of induced mutation than did an *SUQ5 ade2-1 RAD*⁺ [*psi*⁺] strain 507-4/2b (Table 2). All classes of mutation were induced in MT209/7b with the majority of mutants (220 of 255, 86.3%) giving rise to whole rather than sectored clones. This compared with 68.7% whole mutant clones (81/118) induced by UV in 507-4/2b over the same dose range. The effect of *SUQ5* on mutation induction is not likely to be due to selection since the nonsuppressed mutants are more sensitive to UV than the suppressed cells (see above).

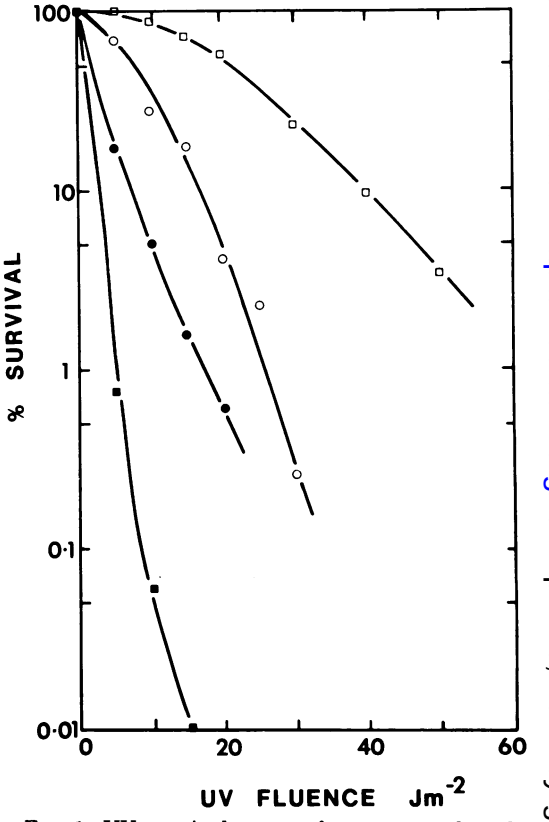

FIG. 1. *UV survival curves for suppressed and nonsuppressed rad6-1 strains and a suppressed RAD*⁺ *strain. Symbols:* ■, *197/2d, sup*⁺ *rad6-1 [psi⁻];* ○, *MT209/7b, SUQ5 rad6-1 [psi⁺];* ●, *MT209/7b, SUQ5 rad6-1 [psi⁻]; and* □, *466/4a, SUQ5 RAD*⁺ *[psi⁺].*

We conclude from these results that both the UV sensitivity phenotype and UV immutability phenotype of *rad6-1* are ochre suppressible. Previous data (11, 13) have shown that *rad6-1* is also suppressible by both amber and omnipotent

TABLE 2. *Induction of nonsuppressed mutants by UV light in SUQ5 ade2-1 [psi⁺] strains that are either RAD⁺ (507-4/2b), rad6-1 (MT209/7b), or rad6-3 (MT221/1b)*

UV dose (J/m²)	*RAD⁺* (507-4/2b)		*rad6-1* (MT209/7b)		*rad6-3* (MT221/1b)	
	Colonies scored[a]	Mutant frequency/10⁻¹ survivors	Colonies scored[a]	Mutant frequency/10⁻¹ survivors	Colonies scored[a]	Mutant frequency/10⁻¹ survivors
0	17,642 (100)	0.17	3,906 (100)	0.26	3,544 (100)	0
10	8,617 (93)	1.51	6,520 (58)	1.53	631 (95)	0
20	11,739 (74)	2.13	7,532 (18)	2.52	5,922 (64)	0
30	1,964 (38)	4.07	5,573 (7)	6.28	ND[b]	
40	9,214 (36)	5.21	6,041 (2)	7.11	1,612 (16)	0
50	8,742 (30)	6.29	6,221 (0.3)	10.45	ND	
60	4,306 (13)	6.73	7,625 (0.05)	10.74	1,131 (2)	0.88
80	10,385 (4)	7.13	ND		7,484 (0.8)	0.94
100	6,188 (0.7)	9.37	ND		10,683 (0.3)	0.94

[a] Percent survival within parentheses is the average of at least two experiments.
[b] ND, Not determined.

translational suppressors. The *rad6-1* mutation thus represents a rare class of mutation in *S. cerevisiae* that is susceptible to both ochre and amber translational suppressors.

Suppression of *rad6-3*. We also examined the ability of *SUQ5* to suppress a second allele of *RAD6⁺*, *rad6-3*. This mutant, originally isolated by Prakash and Prakash (18), has the same pleiotropic phenotypes as the *rad6-1* mutation. As observed with the *SUQ5 rad6-1* [*psi⁺*] strain, MT221/1b (*SUQ5 rad6-3* [*psi⁺*]) showed a marked increase in UV resistance, compared with its *sup⁺ rad6-3* parent JF9-4D (Fig. 2), although it was still less resistant than its *RAD⁺ SUQ5* [*psi⁺*] parent 507-4/2b. However, MT221/2b failed to show any UV-induced mutation in dose ranges where it had been observed for both the *SUQ5 RAD⁺* [*psi⁺*] control and MT209/7b (Table 2). Therefore, only one of the two phenotypes of *rad6-3* examined is ochre suppressible.

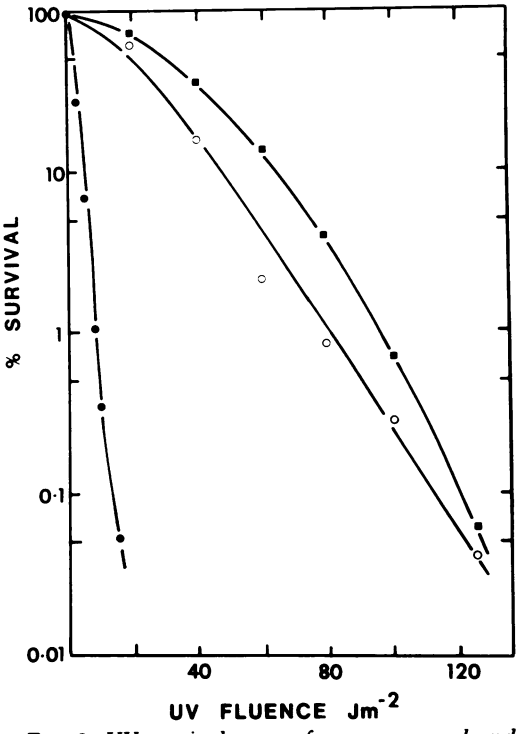

FIG. 2. *UV survival curves for a suppressed and nonsuppressed rad6-3 strain and a suppressed RAD⁺ strain. Symbols: ●, JF9-4D, sup⁺ rad6-3; ○, MT221/1b, SUQ5 rad6-3 [psi⁺]; and ■, 507-4/2b, SUQ5 RAD⁺ [psi⁺].*

DISCUSSION

From the evidence presented here and elsewhere (11, 13), the *rad6-1* mutation is suppressed by both ochre and amber translational suppressors. Very few examples of such mutations are known in *S. cerevisiae* (2, 9). The majority of nonsense mutations described in *S. cerevisiae* are either ochre or amber suppressible. The stringency of ochre suppression in this organism is believed to be due to a modification of the third (U*) base in the suppressor transfer ribonucleic acid anticodon (16). Possibly the context of nucleotides surrounding the nonsense mutant codon in *rad6-1* results in a relaxation of the stringency of the three base codon:anticodon interaction, i.e., "two out of three" reading. The influence of codon context on the efficiency of nonsense suppressors has been well defined in procaryotes (1, 6). The base adjacent to the 3′ side of the nonsense codon plays an important role in the strength of the codon:anticodon interaction (1).

Alternatively, the ability of nonsense suppressors to abolish *rad6* phenotype(s) may not operate at the level of translation but, rather, act indirectly. This seems unlikely, however, be-

cause of the differential effect of *SUQ5* on the phenotypes of *rad6-1* and *rad6-3*. Also the lower UV resistance of an *SUQ5 rad6-1* [*psi*$^-$] strain versus an isogenic [*psi*$^+$] strain (Fig. 1) strongly suggests that suppression is occurring at the level of translation.

The evidence presented here and in several recent publications (13, 14) has suggested that the *RAD6*-dependent functions can be divided into two functionally distinct classes, one concerned with error-free repair, the other with mutagenesis. Lawrence and Christensen (13) have reported on a mutation, *SRS2*, which suppresses the UV sensitivity of *rad6-1* but not its deficiencies with respect to induced mutagenesis or sporulation. *SRS2* is not a translational suppressor. It is worth noting that both translational and metabolic suppression can produce strains resistant to mutation by UV which are, nevertheless, no different from the wild type in UV survival. This suggests that the mutagenic pathway of *S. cerevisiae* contributes little, if anything, to deoxyribonucleic acid repair.

From the current data we would suggest that the *RAD6* gene codes for a multifunctional polypeptide. Multifunctional polypeptides occur in *S. cerevisiae*, with the *HIS4*$^+$ gene being particularly well defined by Fink and co-workers (10). The *HIS4*$^+$ gene codes for a single 95,000-molecular-weight polypeptide with three distinct enzymatic activities. Genetic complementation of *his4* mutants has defined three cistrons (*his4A*, *B*, and *C*), and, because nonsense mutations within *HIS4*$^+$ gene show a polar effect on these three activities, it is deduced that the *HIS4*$^+$ gene is transcribed *ABC* as a single unit.

A model for the organization of the *RAD6*$^+$ gene is given in Fig. 3. The gene is divided into two cistrons, *RAD6A* and *RAD6B*. *RAD6A* codes for an activity responsible for the error-prone repair of UV-induced lesions in deoxyribonucleic acid, but is not involved in a cell's resistance to the lethal efffects of UV. *RAD6B* codes for an activity essential for the error-free repair of potentially lethal mutagenic damage. The two cistrons are transcribed as a single unit, *AB*. *rad6-1* is a nonsense mutation in *RAD6A* having a polar effect and giving an Rad6A$^-$6B$^-$ phenotype. Translational suppression would restore both activities. *rad6-3* would be similar to *rad6-1*, but in this case, the amino acid inserted at this site by the suppressor *SUQ5* (serine) would be incompatible with *RAD6A* function, but would be sufficient to relieve polarity giving an Rad6A$^-$6B$^+$ phenotype.

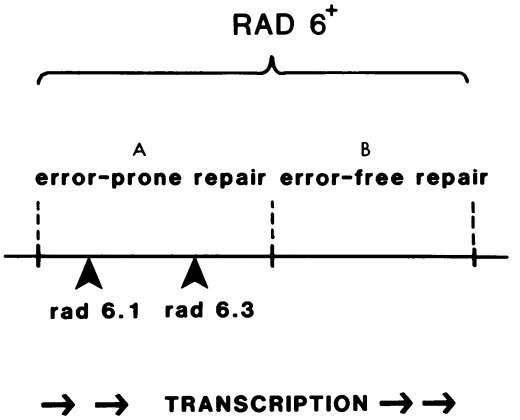

FIG. 3. *A model for the structure of the RAD6*$^+$ *gene.*

The *rad6-1* mutant has three further phenotypes: it gives the cell an increased sensitivity to the antifolate drug trimethoprim (7), diploids homozygous for *rad6-1* fail to sporulate (5), and *rad6-1* strains show an elevated spontaneous mutation frequency (8). The effect of translational suppressors on these phenotypes in both *rad6-1* and *rad6-3* strains is currently under investigation, in hopes of defining which of the two cistrons (or more) code for the activities involved in these processes.

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