ULTRAVIOLET MUTAGENESIS STUDIES OF *[psi]*, A CYTOPLASMIC DETERMINANT OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

UV mutagenesis was used to probe the molecular nature of *[psi]*, a non-mitochondrial cytoplasmic determinant of *Saccharomyces cerevisiae* involved in the control of nonsense suppression. The UV-induced mutation from *[psi⁺]* to *[psi⁻]* showed characteristics of forward nuclear gene mutation in terms of frequency, induction kinetics, occurrence of whole and sector mutant clones and the effect of the stage in the growth cycle on mutation frequency. The involvement of pyrimidine dimers in the premutational lesion giving the *[psi⁻]* mutation was demonstrated by photoreactivation. UV-induced damage to the *[psi]* genetic determinant was shown to be repaired by nuclear-coded repair enzymes that are responsible for the repair of nuclear DNA damage. UV-induced damage to mitochondrial DNA appeared to be, at least partly, under the control of different repair processes. The evidence obtained suggests that the *[psi]* determinant is DNA.

THE cytoplasmically inherited *[psi]* factor of *Saccharomyces cerevisiae* is a potentiator of nonsense suppression. In a *[psi⁺]* background, the serine-inserting ochre suppressor *SUQ5* efficiently suppresses a variety of nonsense mutations, while in a *[psi⁻]* background *SUQ5* is inactive and ochre mutations remain unsuppressed (Cox 1965, 1971; LIEBMAN, STEWART and SHERMAN 1975). Since its discovery in 1965 (Cox 1965) the molecular nature of the *[psi]* determinant has remained an enigma.

The *[psi]* determinant is not associated with the mitochondrial *[rho]* genome (YOUNG and COX 1972; M. F. TUIITE, unpublished results), nor is it associated with the M double-stranded RNA species responsible for the cytoplasmically inherited "killer" phenotype (LEIBOWITZ and WICKNER 1977; COX *et al.*, in preparation). *[Psi⁺]* strains occur that do not have these species of nucleic acid. *[Psi⁻]* strains exist that contain both LdsRNA species and "2 μ " circles. The *[psi⁻]* condition is not, therefore, a consequence of loss of one of these cytoplasmically located nucleic acid species.

Since there is no clear evidence from biochemical studies that demonstrates the biochemical nature of the *[psi]* determinant, we have asked the question whether mutation from *[psi⁺]* to *[psi⁻]* can be caused by standard mutagens known to interact directly with nucleic acid.

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The principal biological effects of UV (254 nm radiation) are a result of damage produced in DNA. The dimerization of adjacent pyrimidines in the DNA gives rise to the principal photo-product, the pyrimidine dimer (PD). In yeast, UV is known to produce pyrimidine dimers in both nuclear and mitochondrial DNA (UNRAU, WHEATCROFT and COX 1971; WATERS and MOUSTACCHI 1974; PRAKASH 1975) and can induce both nuclear and cytoplasmic gene mutations (PATRICK, HAYNES and URETZ 1964; PARRY and COX 1965; MOUSTACCHI 1969; STUART 1970). UV-induced pyrimidine dimers are formed in DNA in proportion to dose (UNRAU *et al.* 1973).

Although UV, like other nuclear mutagens such as ethyl methanesulphonate and nitrosoguanidine, can cause mutation from $[psi^+]$ to $[psi^-]$ (TUIITE 1978), this in itself is not a convincing demonstration that the determinant of $[psi]$ is DNA or even nucleic acid. These agents are far from specific and are likely to cause chemical changes in cell components other than nucleic acid. Cells do, however, contain agents that are highly specific in interacting with DNA; these are the repair enzymes. The substrate for one of these, photoreactivating enzyme, is double-stranded DNA containing pyrimidine dimers (RUPERT 1964; SETLOW and CARRIER 1964). The partially purified enzyme from yeast is, apparently, inactive with dimers in single-stranded RNA (HURTER *et al.* 1974). This does not rule out the possibility that one or more enzymes exist in yeast that have a photolytic action on dimers in RNA, as they do in plants and insects (HUANG and GORDON 1972; HURTER *et al.* 1974; JACKLE and KALTHOFF 1978); if they exist in yeast, though, they seem to contribute nothing to the photoreactivability of survival (RESNICK 1969). Although it has not yet been demonstrated, it is likely that the enzymes of the excision-repair pathway in yeast have a similar specificity and that the same applies to the third major UV-repair pathway in yeast, the so-called "*rad18*" pathway (GAME and COX 1973; COX and GAME 1974). If it can be demonstrated that these repair functions have an effect on the UV-induced mutation of $[psi]$, it would be difficult to avoid the conclusion that DNA is involved in its determination.

In this paper, we describe the kinetics of induction of the $[psi^-]$ mutation by UV and the effects of photoreactivation on it. We also describe the kinetics of induction of the $[psi^-]$ mutation by UV in a number of mutants of yeast defective in repair or in mutagenesis. The mutation from $[psi^+]$ to $[psi^-]$ is compared with the induction of nuclear mutations and of the cytoplasmic *petite* mutation.

MATERIALS AND METHODS

Strains: Strains of *Saccharomyces cerevisiae* used in this study had the general genotype *SUQ5 ade2-1 his5-2 ura3-1 radx [psi^+]* and were constructed from the strains given in Table 1, using standard techniques of yeast genetics (MORTIMER and HAWTHORNE 1969.)

Media: YC (yeast complete) is a rich growth medium described by COX and BEVAN (1962). YNB is a minimal synthetic medium (0.67% Difco yeast nitrogen base without amino acids, 1% glucose, 1.25% agar). YNB was suitably supplemented so as to support growth of auxotrophic strains or to score auxotrophic requirements. Amino acids, uracil and adenine were added, when necessary, at 10 µg/ml.

TABLE 1

Original yeast strains from which the strains described in this paper were derived

Strain	Genotype*	Source
507-4/2b	α <i>SUQ5 ade2-1 his5-2 can1-100 ura3-1</i> [<i>psi</i> ⁺]	B. S. COX
468/1a	a <i>SUQ5 ade2-1 lys1-1 can1-100 ura3-1</i> [<i>psi</i> ⁻]	B. S. COX
466/4a	a <i>SUQ5 ade2-1</i> [<i>psi</i> ⁺]	B. S. COX
X1687-16C	a <i>arg4-17 ade2-1 his5-2 lys1-1 trp5-48</i> [<i>psi</i> ⁻]	F. ECKARDT
478/1b	α <i>ade2-1 trp5-u9 rad1-1</i> [<i>psi</i> ⁻]	B. S. COX
197/2d-u11	α <i>ade2-1 rad6-1</i> [<i>psi</i> ⁻]	B. S. COX
XY-225-6A	α <i>arg4-17 rev3-1</i> [<i>psi</i> ⁺]	J. F. LEMONTT
XY-492-2A	α <i>his5-2 lys1-1 ura4-1 umr1-1</i> [<i>psi</i> ⁺]	J. F. LEMONTT
JF9-4D	a <i>rad6-3 lys1-1</i> and/or <i>lys2-1 his1-1 trp2 arg4-17</i>	C. J. LAWRENCE
EO12-2C	α <i>ade2-1 rad3-12 aux_n</i>	R. C. VON BORSTEL

* *ade2-1 his5-2 lys1-1 can1-100 trp5-48 arg4-17* and *ura4-1* are ochre suppressible nonsense mutations. *aux_n* indicates undefined auxotrophic requirements.

UV irradiation: Cells were grown either to log phase (greater than 60% budded cells) on YC plates at room temperature or to stationary phase (less than 5% budded cells) in 10 ml liquid YC cultures incubated at 28°. Prior to irradiation, cells were washed once and resuspended in sterile saline (0.85% NaCl). The UV source was a Hanovia IIa mercury discharge tube that emitted light mainly at 254nm. The incident dose rate, determined by a galvanometer attached to a photosensitive head, was adjusted to 0.2 Jm⁻²sec⁻¹ for the *rad1* strain and to either 0.5 or 2.0 Jm⁻²sec⁻¹ for all other strains. Ten ml cell suspensions were irradiated for various periods of time in an open petri dish that was agitated during the irradiation period. The cell titer of the irradiated suspension never exceeded 1 × 10⁷/ml. Irradiated cells were diluted in sterile saline and plated onto YC plates to give between 200 and 600 viable colonies per plate. All plates were incubated at 28° for up to 5 days.

Photoreactivation: Irradiated cell suspensions were exposed for 1 hr in a closed petri dish at room temperature to a 500W Phillips "Photoflood" bulb at a distance of 60 cm. A second petri dish, containing 20 ml of a 5% w/v copper sulphate solution, was placed on top of the cell suspension to prevent overheating.

Analysis of nonsuppressed mutants: Nonsuppressed mutants of strains of the genotype *SUQ5 ade2-1 his5-2* [*psi*⁺] give rise to red colonies on YC plates because the ochre suppressible *ade2-1* mutation is expressed, producing a red pigment caused by the accumulation of 5-aminoimidazole ribotide (SILVER and EATON 1969). To enhance the red pigmentation, colonies on YC plates were stored at 4° for up to 5 days prior to scoring. Complementation analysis used to determine the class of mutation responsible for the nonsuppressed phenotype has been described elsewhere (Cox *et al.* 1980). Although these tests allowed us to distinguish several classes of nuclear mutation leading to loss of suppression (reversion to adenine requirement), in this paper we have pooled the numbers of all such mutations for the purposes of comparison with the cytoplasmic mutation.

Detection of petite colonies: YC plates with between 100 and 300 colonies were replica-plated onto YEPG medium (2% glycerol, 1% yeast extract, 1% bacto-peptone, 1.25% agar) and the plates incubated at 28° for 4 days. *Petite* mutants fail to grow on this nonfermentable medium. Only wholly *petite* colonies were scored.

RESULTS

Induction of [*psi*⁻] mutants by UV: The frequencies of nonsuppressed mutants induced by UV in the repair-proficient strain 507-4/2b were measured over a

dose range of 20 to 200 Jm^{-2} . Stationary-phase cells were more sensitive to the lethal effects of UV than were log-phase cells (Figure 1). Stationary-phase cells also gave a higher frequency of nonsuppressed mutants per survivor than log-phase cells at a given UV dose (Table 2).

Approximately 40 nonsuppressed mutants obtained at each dose were classified by complementation analysis (Table 2). All predicted classes of nuclear mutation were found, together with cytoplasmic $[\psi^-]$ mutants. The log-log plots for both the $[\psi^-]$ and nuclear mutations are shown in Figure 2. Both nuclear and $[\psi^+]$ to $[\psi^-]$ mutation show linear induction kinetics in both log- and stationary-phase cells (ECKARDT and HAYNES 1977b).

Photoreactivation studies: Exposure of UV-irradiated yeast cells to white light promotes an increase in cell survival and a reduction in the frequency of both nuclear and cytoplasmic mutations (PATRICK, HAYNES and URETZ 1964; PARRY

FIGURE 1.—The survival after UV of log-phase (O) and stationary-phase (●) cells of the $\text{RAD}^+[\psi^+]$ strain 507-4/2b.

TABLE 2

The numbers of UV-induced nonsuppressed mutants in the RAD⁺ strain 507-4/2b and results of complementation analysis of some of the mutants obtained

	UV dose (Jm ⁻²)	Colonies counted	% Survival	Non- suppressed mutants	Frequency of nonsuppressed mutants × 10 ⁸	Number analyzed	[psi ⁻]	Nuclear
Stationary phase	0	17672	100	3	0.17	3	0	3
	20	11739	74.3	25	2.13	21	7	14
	25	4096	67.6	13	3.17	13	4	9
	40	9214	36.4	48	5.21	40	8	32
	50	8742	30.4	55	6.29	42	11	31
	60	4306	13.2	29	6.73	44*	8	36
	80	10385	4.0	74	7.13	70	10	60
	100	6188	0.66	58	9.37	42	8	34
Log phase	125	2774	0.06	28	9.74	18	6	12
	150	2964	0.03	30	10.12	25	7	18
	0	11377	100	3	0.26	3	2	1
	25	2285	74.0	7	3.06	7	1	6
	50	7374	49.4	26	3.53	24	4	20
	75	8210	29.4	35	4.26	27	5	22
	100	8282	9.1	48	5.80	7	4	33
	150	3102	0.61	25	8.06	70*	10	60
	200	7575	0.14	75	9.90	60	9	51

* Further mutants were obtained for analysis by plating at high cell densities (approx. 10⁸ colonies per plate).

and Cox 1965; MOUSTACCHI and ENTERIC 1970). This photoreactivation (PHR) is mediated by an enzyme, DNA photolyase, that monomerizes pyrimidine dimers *in situ*. The removal of some biological effect by PHR indicates that PD's are involved (SETLOW and CARRIER 1964). We therefore set out to demonstrate their involvement in the premutational event giving rise to the [psi⁻] mutation.

Using the RAD⁺ strain, 507-4/2b, log-phase cells, irradiated at 200 Jm⁻² (0.11% survival) and stationary-phase cells, irradiated at 125 Jm⁻² (0.06% survival), were exposed to white light for one hour immediately after irradiation. Following treatment with white light, cells were immediately plated. Table 3 summarizes the results obtained from two independent experiments.

Both killing and the frequency of nonsuppressed mutants was significantly reduced following a period of illumination with white light. Photoreversal occurred of both cytoplasmic and nuclear UV-induced mutations. The photoreactivable sectors were similar, being 90% and 77% of induced [psi⁻] mutations in log and stationary phase, respectively, and 72% and 76% of induced nuclear mutations in the same experiments. The photoreactivable sectors of survival were 60% in log-phase and 63% in stationary-phase cells. UV damage to the [psi] determinant is therefore photoreactivable, implying the involvement of pyrimidine dimers in the mutation from [psi⁺] to [psi⁻].

FIGURE 2.—The frequency of nonsuppressed mutants as a function of UV dose in the *RAD*⁺[*psi*⁺] strain 507-4/2b, showing nuclear mutations in stationary phase (○), in log phase (●) and [*psi*⁻] mutation in stationary phase (◇) and in log phase (◆).

[*Psi*] *mutagenesis in strains defective in nuclear DNA repair*: Genetic evidence has demonstrated that lesions induced in yeast by UV are subject to different modes of repair (COX and GAME 1974; LAWRENCE and CHRISTENSEN 1976). Some loci are primarily responsible for dimer excision, which, like excision in *E. coli*, is apparently "error-free." The effect of a mutation blocking this DNA

TABLE 3

*The effects of post-irradiation exposure to white light on cell survival and the numbers of nonsuppressed, of [*psi*⁻] and of nuclear mutants*

Treatment		% Survival	Number of colonies counted	Total number of nonsuppressed mutants	Number analyzed	Numbers of [<i>psi</i> ⁻] Nuclear	
Log phase	UV	0.11	4237	52(12.27)	46	7(1.87)	39(10.4)
	UV + PHR	6.62	4871	15(3.08)	61*	3(0.15)	58(2.93)
Stationary phase	UV	0.06	2305	24(10.41)	18	6(3.47)	12(6.94)
	UV + PHR	4.60	7999	20(2.50)	43*	14(0.81)	29(1.69)

* Further mutants were obtained for analysis by plating at high cell densities (approx. 10⁸ colonies per plate).

Figures in parentheses are the frequencies of mutants of each class per thousand surviving cells.

repair pathway is to make the cells very much more sensitive to UV in terms of both survival and mutagenesis. The latter effect is probably due to the fact that, in the absence of excision-repair, pyrimidine dimers are mostly repaired or metabolized by an alternative error-prone pathway. The effect is that the rate of UV-induced mutation, as a function of UV dose, is much higher than it is in wild-type cells. This effect has been demonstrated by assaying reverse mutation in the excision-defective mutants *rad1*, *rad2*, *rad3* and *rad4* (HUNNABLE 1972; LAWRENCE and CHRISTENSEN 1976).

In mutants affecting the other mode of repair, the majority of lesions are presumably repaired by error-free excision repair. Some of these mutants, notably *rad6* and *rev3*, show a marked reduction in UV mutagenesis (LAWRENCE and CHRISTENSEN 1976). In addition, mutants have been isolated that are similar in that they block UV mutagenesis, but show little UV sensitivity. One such mutant is *umr1* (LEMONTT 1977).

We undertook a study of UV-induced [*psi*⁻] mutations in strains defective in one or other of the two major dark-repair pathways to determine whether UV-induced lesions in the [*psi*⁻] genetic determinant were repaired by pathways responsible for the repair of nuclear DNA damage.

Mutants defective in dimer excision: Induction of the [*psi*⁻] mutation by UV was examined in log-phase cells of two *rad1-1* [*psi*⁺] strains; MT141-2/4b (α *SUQ5 ade2-1 his5-2 ura3-1 rad1-1* [*psi*⁺]) and MT141-4/6d (α *SUQ5 ade2-1 his5-2 ura3-1 rad1-1* [*psi*⁺]) over a dose of 0 to 10 Jm⁻². Both strains were significantly more UV sensitive than the *RAD*⁺ strain, 507-4/2b (Figure 3). Table 4 summarizes our results obtained with the two *rad1-1* strains from four independent experiments for each strain. The frequency of nonsuppressed mutants was significantly enhanced in the *rad1-1* strains. No significant induction of nonsuppressed mutants was seen at the same UV doses in the *RAD*⁺ control.

A number of mutants of MT141-4/6 were analyzed by complementation; Table 4 gives the number of [*psi*⁻] and nuclear mutants obtained. A log-log plot (Figure 4) shows clearly that the frequency of both nuclear mutations and that of the [*psi*⁻] mutation are a factor of 10 higher than that in the *RAD*⁺ control. As was observed in the *RAD*⁺ 507-4/2b strain, the induction of these mutations followed single-hit kinetics.

Results obtained with a *rad3-12* strain (MT220/8c = α *SUQ5 ade2-1 his5-2 ura3-1 rad3-12* [*psi*⁺]) over the dose range 0 to 40 Jm⁻² demonstrated that, although slightly more UV resistant than the *rad1-1* strains, it shows enhanced UV mutagenesis (Figures 3 and 4). Of 58 MT220/8c mutants analyzed following a UV dose of 20 Jm⁻², 24 were [*psi*⁻] (frequency 3.84×10^{-3} survivors). The frequency of nuclear mutations was 5.4×10^{-3} survivors. Of 25 nonsuppressed mutants obtained at this dose with the *RAD*⁺ strain 507-4/2b, seven were [*psi*⁻] (frequency 0.71×10^{-3} survivors) with the frequency of nuclear mutations being 1.42×10^{-3} survivors.

In both the *rad1-1* and the *rad3-12* strains, the frequency of nonsuppressed mutants of both nuclear and cytoplasmic origin was higher than in the *RAD*⁺ strain at equivalent UV fluences. This suggests that lesions induced in the [*psi*]

FIGURE 3.—The survival after UV of excision-defective [*psi*⁺] strains used in this study compared to the excision-proficient *RAD*⁺ [*psi*⁺] strain 507-4/2b. Symbols are: 507-4/2b *RAD*⁺ (○), MT141-2/4b *rad1*-1 (●), MT141-4/6d *rad1*-1 (◆), MT220/8c *rad3*-12 (◇).

genetic determinant are subject to repair by the same excision repair pathway as are nuclear determinants, and also by an error-prone pathway.

Mutants defective in the rad18 ("error-prone") pathway: UV-induction of the [*psi*⁻] mutation was measured in [*psi*⁺] strains carrying mutations that have been found to block error-prone repair. These were *rev3*-1 (LEMONTT 1971), *umr1*-1 (LEMONTT 1973) and *rad6*-3 (PRAKASH and PRAKASH 1977).

Rev3-1 causes a reduction in UV-induced reversion of the highly UV-reversible ochre allele, *arg4*-17 (LEMONTT 1971). The *REV3*⁺ gene product appears to be necessary for all types of UV mutagenesis at all sites within the nuclear genome (LAWRENCE and CHRISTENSEN 1976). [*Psi*] mutagenesis was measured in two different *rev3*-1 [*psi*⁺] strains, BSC494/5a (*α* *SUQ5 ade2*-1 *his5*-2 *lys1*-1 *trp5*-48 *rev3*-1 [*psi*⁺]) and MT196/3a (*α* *SUQ5 ade2*-1 *his5*-2 *can1*-100 *rev3*-1

TABLE 4
The numbers of UV-induced nonsuppressed mutants in RAD⁺ and rad1 [psi⁺] strains and the distribution among them of [psi⁻] and nuclear mutations

UV dose (Jm ⁻²)	RAD ⁺					rad1				
	Colonies counted	Nonsuppressed mutants	Mutant frequency X 10 ³	[psi ⁻]	Nuclear	Colonies counted	Nonsuppressed mutants	Mutant frequency X 10 ³	[psi ⁻]	Nuclear
0	11377	3	0.26	2	1	4846	7	1.44	0	3
1	1610	0	0			3125	13	4.16		
2	4078	2	0.49	0	2	10745	55	5.12	3	16
3	1639	0	0			785	5	6.37		
4	3810	2	0.52	0	2	11608	95	8.18	6	55
5	1575	0	0			2169	23	10.60		
6	3796	1	0.26	0	1	18624	200	10.74	8	41
8	2170	3	1.38	0	3	3201	45	14.06	3	18
10	2247	1	0.44	0	1	4642	66	14.22	3	16

FIGURE 4.—The frequency of nonsuppressed mutants as a function of UV dose in excision-deficient [*psi*⁺] strains. Symbols are: nuclear mutations in *rad1-1* (□); [*psi*⁻] mutations in *rad1-1* (■) and total nonsuppressed revertants in *rad3-12* (●). ---- represents nuclear mutations in stationary phase and —·— represents [*psi*⁻] mutations in stationary phase of *RAD*⁺ strains (see Figure 2).

[*psi*⁺] over a dose range of 0 to 100 Jm⁻². The former was markedly more UV resistant than the latter strain (Figure 5) and modification of the UV-sensitive phenotype probably occurred during its storage on YEPD at 4° for two years prior to these experiments. MT196/3a was constructed immediately prior to these experiments and showed the predicted UV-sensitive phenotype (Figure 5).

Mutagenesis was performed with stationary phase cells. The data obtained from a number of independent experiments are summarized in Table 5. Compared to the *RAD*⁺ strain 507-4/2b, at equivalent UV doses, the frequency of nonsuppressed mutants was reduced. Twenty-four of the 32 nonsuppressed *rev3-1* mutants obtained were characterized: one was a [*psi*⁻] mutant, the remainder nuclear mutations.

Umr1-1 is one of the seven nonallelic mutants that show a reduced capacity for UV-induced forward mutation from *CAN*⁺ to *can1* (LEMONTT 1973). *Umr1-1* mutants, unlike *rev* or *rad* mutants, are not significantly UV-sensitive (Figure 5; see also LEMONTT 1977). They show a reduced frequency of UV-induced reversion of some, but not all, of the ochre alleles tested (LEMONTT 1977). The phenotype of *umr1-1* mutants is consistent with the *UMR*⁺ gene lying in the "rad18" pathway or at least a branch of that pathway required for specific mutational events.

[*Psi*] mutagenesis was examined in stationary phase cells of two different *umr1-1* [*psi*⁺] strains MT195/1b (*α* *SUQ5 ade2-1 his5-2 lys1-1 ura3-1 umr1-1*

FIGURE 5.—Survival curves of 507.4/2b (RAD^+) (○); two different *rev3-1* strains, 494/5a, (□) and MT196/3a (■); MT195/1b (*umr1-1*) (●) and suppressed and unsuppressed *rad6-3* strains, MT221/1b, *rad6-3* *SUQ5*[*psi*⁺] (△) and JF9-4D *rad6-3* *suq5*⁺ (▲).

[*psi*⁺]) and MT195/2a (a *SUQ5 ade2-1 his5-2 lys1-1 ura3-1 umr1-1* [*psi*⁺]) over a dose range of 0 to 100 Jm^{-2} . Table 5 summarizes the data we obtained in a number of independent experiments conducted with these two strains. It is evident that, at equivalent UV doses, the frequency of nonsuppressed mutants in the *umr1-1* strains was significantly less than that observed in the RAD^+ strain 507-4/2b. Of 62 *umr1-1* nonsuppressed mutants analyzed, eight were [*psi*⁻] and 54 were nuclear mutations.

Rad6-3: The $RAD6^+$ gene, which appears to control a step in the error-prone pathway (Cox and GAME 1974), codes for a gene product that is apparently required for all kinds of induced mutagenic events at all sites within the nuclear genome so far tested (PRAKASH 1974; LAWRENCE and CHRISTENSEN 1976). UV mutagenesis was examined in the strain MT221/1b (a *SUQ5 ade2-1 his1-1 ura3-1 rad6-3* (*his5-2 can1-100 arg4-17*[±]) [*psi*⁺]). This strain was only slightly UV-sensitive compared to the RAD^+ strain 507-4/2b (Figure 5), but the *rad6-3* parent strain JF9-4D (a *rad6-3 lys1-1* and/or *lys2-1 his1-1 arg4-17*

TABLE 5
A comparison of the frequencies of nonsuppressed revertants in various strains blocked in error-prone repair of UV damage

UV dose (Jm ⁻²)	rev1-3			umt1-1			rad6-3		Stationary phase RAD+ UMR+ frequency × 10 ⁻⁸
	Colonies counted	Non- suppressed revertants	Frequency × 10 ⁻⁸	Colonies counted	Non- suppressed revertants	Frequency × 10 ⁻⁸	Colonies counted	Non- suppressed revertants	Frequency
0	14525	1	0.07	6545	0	—	3544	0	—
5	4006	1	0.25						
10	6283	1	0.16				631	0	—
15	5082	5	0.98						
20	9640	9	0.93	7014	6	0.76	5922	0	—
25	3245	1	0.31						2.13
40	4112	3	0.73	6816	5	0.73	1612	0	—
50	5978	3	0.50						3.17
60	4507	2	0.44	8526	7	0.82	1311	1	0.88
80	11377	2	0.18	7736	6	0.78	7484	7	0.94
100	8471	4	0.47	18021	25	1.39	10683	10	0.94
Number of revertants analyzed		24			62			6	See Table 2.
Number of [psi-]		1			8			0	

trp2) was significantly more UV-sensitive than either of these two strains (Figure 5). We have shown that the resistance to killing by UV of MT221/1b is due to the ochre suppressor *SUQ5* (M. Tuite and B. S. Cox, unpublished).

UV irradiation of stationary phase cells of MT221/1b, over the dose range of 0 to 100 Jm⁻², was carried out. The data we obtained from three independent experiments are summarized in Table 5. The frequency of nonsuppressed mutants obtained was significantly less, at a given UV dose, than that observed for the *RAD*⁺ strain 507-4/2b. All six nonsuppressed mutants analyzed were nuclear mutations; none was [*psi*⁻].

We have also measured UV mutagenesis in a strain of genotype *SUQ5 ade2-1 rad6-1* [*psi*⁺]. This allele of *rad6* also shows a slightly enhanced UV resistance due to the presence of the ochre suppressor, but considerably enhanced UV-induced mutation frequencies, including that of the [*psi*⁻] mutation (M. Tuite and B. S. Cox, unpublished). The *rad6-1* allele was previously reported to be amber suppressible (Lawrence *et al.* 1974) and, therefore, appears to represent a new class of yeast nonsense mutation, namely, suppressible by both ochre and amber translational suppressors. The results of this study of suppressibility of alleles of *rad6* will be reported in detail elsewhere.

In summary, the frequency of UV-induced, nonsuppressed mutations, including the [*psi*⁻] mutation, is depressed in strains that are defective in error-prone repair or in mutagenesis as judged by mutation of chromosomal markers. This suggests that lesions induced by UV in the [*psi*⁻] genetic determinant are subject to repair by the same error-prone pathways as are those in nuclear determinants.

UV-induction of the cytoplasmic petite mutation: MOUSTACCHI (1969, 1972) originally investigated the UV induction of the cytoplasmic *petite* mutation in a number of repair-deficient mutants. She concluded that the gene products of at least some (but not all) of the *RAD*⁺ genes, *e.g.*, *RAD2*, *RAD6*, *RAD18*, are involved in the repair and/or expression of mitochondrial DNA damage induced by UV light.

The kinetics of induction of the cytoplasmic *petite* mutation by UV light in the *RAD*⁺ strain 507-4/2b was investigated in both log- and stationary-phase cells. In both cases, higher-order kinetics of induction was observed (Figure 6), in contrast to the linear kinetics of induction of both nuclear and [*psi*⁻] mutation in this strain over the dose range studied (see Figure 2). The frequency of *petites*, per unit dose of UV, was enhanced in stationary phase cells compared to log-phase cells, as observed with nuclear and [*psi*⁻] mutation in the same strain.

In the two excision-defective [*psi*⁺] strains, MT141-4/6d (*rad1-1*) and MT220/8c (*rad3-12*), the frequency of *petite* mutants was significantly higher at a given UV fluence than in the excision-proficient (*RAD*⁺) strain 507-4/2b (Figure 7). However, a large number of nuclear genes are required to maintain growth on nonfermentable carbon sources such as glycerol. Mutations in nuclear genes give rise to the so-called "segregational *petites*" (Lachowicz *et al.* 1969). The observed increase in the frequency of *petites* in both the *rad1-1* and *rad3-12*

FIGURE 6.—The frequency of *petite* mutants as a function of UV dose in log- and stationary-phase cells of the *RAD*⁺ [*psi*⁺] strain 507-4/2b. Symbols are: stationary phase (●), log phase (○). The spontaneous *petite* frequency in each case has been subtracted from all values. Each point represents data from an average of 1000 colonies. ---- represents nuclear mutations; -·- [*psi*⁻] mutations in stationary phase cells of 507-2/4b (both × 10) (see Figure 2).

strains may reflect the increase in nuclear gene mutation rather than in cytoplasmic mutation.

Both the *umr1-1* [*psi*⁺] strain MT195/1b and the *rev3-1* [*psi*⁺] strain MT196/3a were found to be hypersensitive to *petite* induction by UV light (Figure 8). A second *umr1-1* [*psi*⁺] strain MT195/2a showed a response similar to that of the *RAD*⁺ strain (data not shown). The possibility that MT196/3a carried a nuclear mutation, unlinked to *rev3-1*, conferring the hypersensitive *petite* induction phenotype was not ruled out. A nuclear mutation *uvs rho5*, conferring such hypersensitivity to *petite* induction by UV, has recently been reported (MOUSTACCHI, PERLMAN and MAHLER 1976). It is clear from the data presented that both MT195/1b and MT196/3a are defective in mitochondrial-

FIGURE 7.—The frequency of *petites* as a function of UV dose in excision-deficient [*psi*⁺] strains compared to the *RAD*⁺ [*psi*⁺] strains 507-4/2b. The symbols are: (a) MT141-4/6d *rad1-1* (■), 507-4/2b *RAD*⁺ (□). (b) MT220/8c *rad3-12* (●), 507-4/2b *RAD*⁺ (○). The spontaneous frequency in each case has been subtracted from all values. Each point represents data from at least 1000 colonies.

specific repair pathway(s), but this has no effect on [*psi*] or nuclear UV mutagenesis.

The *rad6-3* [*psi*⁺] strain MT221/1b was a *petite*. Induction by UV of the *petite* mutation was investigated in a nonsuppressed *rad6-1* strain MT209/7b (α *SUQ5 ade2-1 his5-2 rad6-1* [*psi*⁻]). This strain gave a slightly higher frequency of *petite* induction than the *RAD*⁺ strain 507-4/2b over the dose range measured (Figure 8). This differs from the observations of MOUSTACCHI (1972) who reported that the *rad6-1* mutation decreased *petite* induction by UV.

FIGURE 8.—The frequency of *petites* versus UV dose in strains blocked in UV-induced nuclear mutagenesis compared to that in the *RAD*⁺ [*psi*⁺] strain 507-4/2b. Symbols are: 507-4/2b *RAD*⁺ (■), MT195/1b *umr1-1* (○), MT 196/3a *rev3-1* (□), MT209/7b *rad6-1* (●). The spontaneous frequency in each case has been subtracted from all values. Each point represents data from an average of 1000 colonies.

These results confirm that the repair of UV-induced damage to the mitochondrial genome is, at least partly, under genetic control different from the repair of either the nuclear genome or the [*psi*] genetic determinant.

DISCUSSION

*Molecular nature of the [*psi*] determinant:* Analysis of the response to mutagens with a known effect on DNA is one of the most powerful methods of studying the nature of genetic material at the cellular level. Such an analysis, using UV light to induce the cytoplasmic [*psi*⁻] mutation, was undertaken as part of our studies on the molecular nature and location of the [*psi*] genetic determinant.

Several lines of evidence argue in favor of the [*psi*] phenotype being coded for by a DNA genetic determinant: (1) The kinetics of induction of the [*psi*⁻] mutation is identical to that observed for nuclear forward mutation in the same strain. (2) Both [*psi*] and nuclear mutagenesis show similar growth-phase modifications of mutation frequency, with log-phase cells showing lower mutation frequencies than stationary-phase cells. (3) [*psi*] mutants are picked up as both whole and sectorial mutant clones following mutagenesis. In stationary-phase cultures of the *RAD*⁺ strain 507-4/2b, 57 (70.4%) of the [*psi*⁻] mutants arose as whole mutant clones and 24 (29.6%) as sectorial mutant clones. This

is comparable with nuclear mutations scored in the same experiments; 215 (66.8%) arose as whole mutant clones and 107 (33.2%) as sectorized mutant clones. In stationary phase cells, therefore, the premutational lesion giving rise to the $[psi^-]$ mutation, as with nuclear mutation, is more likely to lead to a pure mutant clone. Little is known about pure mutant colony formation in yeast, although recent studies have suggested that conversion of nonmutant:mutant heteroduplexes to mutant:mutant homoduplexes by some mode of DNA repair is an essential feature (HANNAN, DUCK and NASIM 1976; ECKARDT and HAYNES 1977a,b; JAMES and KILBEY 1977; JAMES, KILBEY and PREFONTAINE 1978. (4) UV-induced lesions in the $[psi]$ genetic determinant are photoreactivatable to the same extent as such lesions induced by UV in nuclear DNA. This implicates the formation of pyrimidine dimers in the $[psi]$ genetic determinant as being at least partly responsible for the UV-induced $[psi^-]$ mutation. (5) UV-induced lesions in the $[psi]$ genetic determinant are subject to repair by the *RAD3* excision repair pathway. (6) UV mutagenesis of the $[psi]$ determinant depends on functions coded by the *RAD6*⁺, *REV3*⁺ and *UMR1*⁺ loci. The gene products of these three loci are believed to form part of the error-prone pathway, and these functions are essential for UV mutagenesis of the nuclear genome (LAWRENCE and CHRISTENSEN 1976; LEMONTT 1977).

The quantitative similarities in induction kinetics, in sectoring frequencies and in the modification of cytoplasmic and nuclear mutation frequencies by post-irradiation treatment and, as a result of repair defects, are particularly striking observations.

Unless all three repair systems act on a cytoplasmic RNA determinant to exactly the same degree of efficiency and with the same relative efficiencies as they act on nuclear DNA, it must be concluded that $[psi]$ is determined by DNA.

The number of [psi] determinants: The "single-hit" kinetics of induction of the $[psi^-]$ mutation implies that only one premutational lesion is required for the mutation. A recent study of the kinetics of elimination of the $[psi^+]$ phenotype from a *PNM*⁻/*pnm*⁺ diploid has shown that, presuming $[psi]$ to be an autonomous genetic determinant and that only one $[psi^+]$ determinant is required for the $[psi^+]$ phenotype, approximately 58 $[psi]$ genetic determinants are present in a haploid $[psi^+]$ cell (McCREADY, COX and McLAUGHLIN 1977). Mutation from $[psi^+]$ to $[psi^-]$ would therefore require simultaneous mutation in all 58 molecules. To account for the single-hit kinetics observed one can postulate that: (1) There exists a single "master" copy of the $[psi]$ genetic determinant that would give rise to a number of "slave" $[psi]$ determinants. A specific version of this model, the "reference-copy" hypothesis, has recently been proposed by OLIVER (1977) to account for *petite* mutagenesis in yeast. A haploid yeast strain characteristically contains about 50 mitochondrial "genomes" (WILLIAMSON 1970). Target-theory analysis has suggested that there are a small number (between one and eight) of target sites. OLIVER's model proposes that there are a small number of "reference" copies of mitochondrial DNA present in the cell, and once per cell cycle, all other "derived" copies of mitochondrial DNA

are compared and corrected against a reference copy by a process of asymmetric gene conversion. Such a "reference-copy" hypothesis would explain the observed "single-hit" kinetics for the $[psi^-]$ mutation. A mutation in a single "reference" $[psi^+]$ determinant would give rise exclusively to "derived" mutant $[psi^-]$ determinants.

(2) Alternatively, the $[psi^-]$ mutation arises by an indirect action where mutation in a single $[psi]$ determinant results in the production of an altered repressor that would prevent the expression of a nuclear gene or genes whose products are required for the replication of the $[psi]$ determinants.

The repair of UV-induced cytoplasmic DNA damage: The results presented in this communication demonstrate that the repair of UV-induced mitochondrial DNA damage is partly under the control of mitochondrial-specific repair systems while repair of UV-induced damage to the $[psi]$ genetic determinant is under the specific control of nuclear DNA repair systems. Two strains examined (MT195/1b and MT196/3a) were both hypersensitive to *petite* induction by UV and presumably carried defects in the repair of UV-induced mitochondrial DNA damage (MOUSTACCHI, PERLMAN and MAHLER 1976). Both of these strains, however, were blocked in both $[psi]$ and nuclear mutagenesis. Therefore, it seems that the two cytoplasmic determinants $[rho]$ and $[psi]$ are subject to different repair systems and is further evidence for their nonidentity (YOUNG and COX 1972).

It should be noted that the *petite* mutation is unique and that it is characteristically accompanied by gross physical changes in mitochondrial DNA (see NAGLEY, SRIPRAKASH and LINNANE 1977 for review). The $[psi^-]$ mutation, however, appears to arise by point mutation rather than by gross physical change because the mutation is revertible (YOUNG and COX 1975; M. F. TUIE, unpublished). UV has been shown to induce point mutations in mitochondrial DNA (STUART 1970). An analysis of the effect of nuclear DNA repair-deficient mutants on the frequency of UV-induced point mutation in mitochondrial DNA is therefore required before one can unequivocally state that different repair systems act on the same type of UV-induced damage to DNA in different cytoplasmically located determinants.

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