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GENETIC CONTROL OF LETHALITY AND MUTATION IN *SACCHAROMYCES CEREVISIAE*

Michael A. Resnick

(Ph.D. Thesis)

August 1968

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TABLE OF CONTENTS

		Page
	Abstract	1
I.	Introduction	4
	A. Repair in general	4
	B. Repair in bacteria	6
	C. Repair in yeast	8
	1. Dark repair	8
	2. Photoreactivation	10
	3. Repair and mutation induction in yeast	12
II.	Materials and methods	13
	A. Yeast strains	13
	B. Media	13
	C. Genetic analysis	15
	D. Radiation sources	16
	E. Radiation studies	17
III.	Results: Mutants sensitive to UV and X-rays	19
	A. Isolation of mutants	19
	B. Genetics of mutants	19
	1. Genetic properties of mutants	19
	2. Allelism of mutants	20
	3. Centromere linkage of uvs 9 and uvs 1	27
	4. Suppressibility of mutants	28
	5. Sporulation of radiation sensitive mutants	30
	C. Sensitivity of mutants to X-rays and UV	31
	1. uvs mutants	31
	2. uxs mutants	36

3. xs mutants 36

D. Repair deficiency associated with uvs 9-3 40

1. Recovery after irradiation 40

2. Photoreactivation after incubation in YEPD 42

3. Inactivation by pyrimidine dimers 47

IV. Results: A "photoreactivationless" mutant of yeast 49

A. Introduction 49

B. Procedure 50

C. Results 52

1. Genetics of the phr mutant 52

2. Influence of phr 1 on UV-induced lethality 53

3. Dominance of PHR gene 58

V. Results: Induction of forward mutations and the effects of repair 61

A. Introduction 61

B. Procedure 61

1. Characterization of mutants 61

2. Isolation of mutants 65

C. Results 68

1. Forward mutation induction - uvs strain 68

2. Forward mutation induction - UVS strain 75

3. Sectoring of forward mutants in the uvs and UVS strains 78

VI. Results: UV-induced reversion of mutations and the effects of repair 83

A. Introduction 83

B. Characteristics of mutants employed in reversion studies 84

C. Procedure 89

D. UV-induced reversion in a uvs 9-3 strain 92

E. Repair of premutational damage in a uvs 9-3 strain 111

F. Comparison of UV-induced reversion in a uvs 9-3 and a UVS strain 114

G. Repair of mutational vs. repair of lethal damage 125

H. Induction of super-suppressors 134

VII. Discussion 136

A. Radiation-sensitive mutants 136

B. Model for dark repair in yeast 138

C. Photoreactivation in yeast 145

D. UV-induced mutagenesis and the role of repair 147

1. Induction of forward mutations 147

2. Reversion of mutations 149

E. Relevance of dark repair and photoreactivation in evolution 152

VIII. Summary 155

Acknowledgments 158

IX. References 159

GENETIC CONTROL OF LETHALITY AND MUTATION IN *SACCHAROMYCES CEREVISIAE*

Abstract

Michael A. Resnick

Eight radiation-sensitive mutants of *Saccharomyces cerevisiae* were isolated: three were ultraviolet-light (UV) sensitive; four were X-ray sensitive, and one was UV and X-ray sensitive. Some of the mutations prevented sporulation. The UV-sensitivity genes are centromere-linked. One (*uvs 1*) is 19.5 centimorgans from the centromere of chromosome XVI and the other (*uvs 9*) is 23 centimorgans from the centromere of a newly identified chromosome XVII. Sensitivities and genetics of the mutants were compared to those reported by other investigators. It is concluded that there are at least 13 genes in yeast that affect sensitivity to radiation. Based on these comparisons and experiments, a model is proposed for the repair of radiation damage.

The UV-sensitive mutant, *uvs 9-3*, was tested for liquid holding recovery; increased survival was observed when held in buffer after UV-irradiation. However, there was no change in the amount of lethal damage that could be photoreactivated after liquid holding. When cells were incubated in nutrient medium after being held in buffer, photoreactivability decreased exponentially with time of incubation. The decrease is attributed to the onset of DNA synthesis.

A "photoreactivationless" mutant was identified. The *phr 1* mutation prevented photoreactivation (PR) in UV-sensitive and wild type haploid and diploid strains (when homozygous). For the case of a *PHR 1* diploid photoreactivability was the same whether the diploid was *PHR 1/phr 1* or *PHR 1/PHR 1*.

Forward mutation induction was examined in a UV-sensitive strain (uvs 9-3) and a wild type strain. Mutants at the tr 5 locus were classified according to ability to complement, suppressibility and osmotic remediability. A mutant exhibiting any one of these characteristics was considered to have arisen by a base-pair substitution event. In the uvs 9-3 strain out of 42,233 colonies 16 tr 5 mutants were isolated without PR, while none were isolated after PR (0/23,541 colonies). Twelve of the mutants were attributed to base-pair substitution events; thus, pyrimidine dimers were concluded to cause primarily base-pair substitution mutations at this locus.

Since the mutation frequency at the tr 5 locus in the UVS strain was the same before and after PR, the lesions causing the mutations were considered to be those other than pyrimidine dimers. However, this damage also produced mainly base-pair substitution mutations (16/19).

The production of whole colony and sectored mutants for several loci was also examined in the uvs 9-3 and the UVS strains. It is proposed that whole colony mutants arise by dark repair acting in some way on the DNA strand opposite the mutational lesion.

For the UV-sensitive and wild type strains, induced reversion to prototrophy by UV occurred regardless of whether the original auxotrophic mutation arose by a base-pair substitution or by an addition-deletion event. In all cases tested a significant amount of reversional lesions were due to pyrimidine dimers being induced in the UVS strain, while in the sensitive strain (uvs 9-3) pyrimidine dimer damage could be detected only when reversion occurred by addition-deletion events. At equal doses, UV-induced reversion frequencies in the UVS strain were always less than in the uvs 9-3 strain.

A comparison between dark repair of lethal and mutational damage indicated that lethal lesions are generally repaired more readily than mutational lesions. Similarly, photoreactivation removes lethal damage in the uvs strain more efficiently than mutational damage; however, in the wild type strain both types of damage are removed with equal efficiency.

The relevance of repair in evolution is discussed. It is suggested that there may still be a selective-advantage for those organisms possessing a photoreactivation system although there is no exposure to far UV (<2900 Å) at the earth's surface.

GENETIC CONTROL OF LETHALITY AND MUTATION IN SACCHAROMYCES CEREVISIAE

I. INTRODUCTION

A. Repair in General

Characteristic of living forms is the ability to ward off or inactivate challenges to their propagative and viable nature. Among the challenges probably present during the early stages of evolution were ionizing radiation and far ultraviolet light [(UV, below 3000 Å) (108)]. Rupert and Harm (106) have suggested that UV reactivation mechanisms were developed in response to the far UV incident on the earth's surface during early evolution. The present level of oxygen allows enough ozone in the upper atmosphere to be produced to effectively absorb all UV incident on the earth below 2900 Å (109). The development of a defense system against the inactivating effects of UV and ionizing radiation as well as other mutagens (discussed in Section I. B.) during the evolution of single cells could account for its presence in the cells of many diverse and complex organisms.

The nature of the radiation defense system and the damage produced by radiation has been examined primarily in the prokaryotic organisms. This defense system has been studied with regard to challenges to the heritable component, the DNA of the cell. Challenges to other parts of a cell could result in killing; however, the effect of the non-DNA damage is relatively insignificant when compared to DNA damage (53). Therefore, the defense system against radiation which may be considered of first-order importance for cell survival is that which modifies and/or removes damage in the DNA of a cell, and has been termed "repair".

Although a good deal is known about the repair mechanisms in prokaryotic organisms (for reviews see 131, 115, 53, 56, 121), there has been relatively little investigation of the genetic nature of repair in higher organisms or even phylogenetically simple eukaryotic cells. The yeast Saccharomyces cerevisiae, a simple eukaryote that exists as a haploid and a diploid, constitutes a convenient transitional organism for examination of the generality of repair in eukaryotic and prokaryotic organisms.

The questions concerning repair of potentially lethal and mutagenic damage in yeast that are investigated in the present study are:

1. What types of damage are repaired?
2. What are the mechanisms of repair?
3. Are repair systems in yeast similar to those in bacteria?
4. What is the nature of the genetic control of repair?
5. How does repair differ in diploid and haploid cells?

An advantage of studying mutational in addition to lethal damage is that the molecular effects of premutational lesions are transmitted to succeeding generations. Thus, the nature of an initial molecular change can be deduced by examining the genetic properties of colonies of cells derived from a single mutated cell.

Also relevant to the importance of repair in evolution is whether induced mutations result from a lack of repair or from occasional errors during repair. Mutations are essential raw material for evolutionary change and the improvement of a species. However, since mutations are usually detrimental, a high mutation rate caused by environmental UV- and X-rays during early evolution would probably

lead to extinction. Therefore, it is predicted that the repair mechanism is efficient for repairing different types of induced premutational damage.

B. Repair in Bacteria

Often the existence of a function is demonstrated by changes leading to the absence of this function. The study of repair in bacteria and the demonstration of its genetic control is based largely on the discovery of mutants sensitive to radiation that presumably have a defective repair system. Many such strains of bacteria have been isolated and the corresponding genes characterized and mapped (1).

The actions of the genes controlling radiation sensitivity have been studied extensively. The demonstration that resistance is due to an active repair of DNA (rather than, for example, an inherent difference in DNA sensitivity) has been facilitated by studies of the response of cells to UV-induced pyrimidine dimers in the DNA (4).

At low doses, UV is known to produce *in vivo* cyclobutane-type pyrimidine dimers (125). These lesions, if not removed, contribute to cell killing (115). *In vitro*, such lesions have been shown to alter DNA synthesis (123, 125) and to inactivate transforming DNA (106). Transforming activity can be restored by a photoreactivating enzyme from yeast (103). Photoreactivating enzyme attaches to DNA at the dimerized region and cleaves the dimer *in situ* (16) when the enzyme-dimer complex is exposed to light of about 3600 to 4200 Å. Similarly survival of radiation-sensitive bacteria is greatly enhanced by exposure to photoreactivating light after UV irradiation (122). The increase in survival is associated with a corresponding decrease in pyrimidine dimers. Consequently, the role of one type of UV

induced damage - pyrimidine dimers - can be assessed by determining the amount of damage that can be photoreactivated.

In addition to photoreactivation another efficient mechanism exists for modifying pyrimidine dimers. In a wild type strain of Escherichia coli an average of about 4000 induced dimers per genome is required to prevent reproduction of a cell (57), whereas in a sensitive strain, B_{s-1}, an average of about 2 dimers can prevent reproduction (125). The dimers in the wild type strain have been shown to be removed by a mechanism known as dark repair. Pyrimidine dimer removal has been demonstrated by their disappearance from trichloroacetic acid (TCA) insoluble DNA and their corresponding appearance in a TCA soluble fraction (6). The latter fraction contains oligonucleotides and single bases of DNA. As shown by Pettijohn and Hanawalt (99), non-conservative replication follows such removal.

Current models of DNA repair propose either a "cut and patch" or a "patch and cut" mechanism. The first mechanism (57) involves 1) induction of a break at or near the dimer by an endonuclease, 2) removal of the dimer and some adjacent bases by an exonuclease, 3) replacing the excised DNA using the opposite strand as a template, and 4) joining the old and new DNA by a ligase. The other model (53) has step 1) and step 4) in common. However, steps 2) and 3) occur concomitantly. Further support for such models has been the isolation of a ligase (139) and the demonstration of endonuclease activity in bacterial cell extracts (126).

The repair system in bacteria is general in that it acts not only on UV-induced damage but also on non dimer-type damage produced by such mutagens as nitrogen mustard (135), methylmethanesulphonate

(113), X-rays (52) and nitrous acid (57). Strains sensitive to these agents have also been reported to be defective in some step of the UV repair system. If bacteria did not have a general repair mechanism then it should be possible to isolate mutants sensitive to one type of mutagen but not sensitive to UV. To date, no such mutants have been reported.

C. Repair in Yeast

1. Dark Repair

The existence of genes controlling radiation resistance in yeast has been demonstrated by the isolation of mutants which are sensitive to UV, X-rays, or to both UV and X-rays. If mutants identified by Nakai and Matsumoto (91) and Snow (129) are at different loci, there are at least eight genes that influence sensitivity to UV. In addition two X-ray sensitive mutants that are also UV-sensitive have been reported (73, 91). All of these mutants are recessive. A dominant X-ray sensitive mutant has been reported by Puglisi (100). Diploid strains carrying this mutation do not sporulate while corresponding tetraploid strains do. As noted by Puglisi it is plausible that altered X-ray sensitivity represents a change in chromosome number rather than a genic alteration.

Although the existence of the above mutants demonstrates that radiosensitivity is under genetic control in yeast there is no direct evidence that modification of DNA damage in yeast, or any other fungus, occurs via a dark repair mechanism similar to that reported in bacteria. In fact, one important dissimilarity exists between bacteria and *Saccharomyces cerevisiae*. The X_1^s mutant reported by Nakai and Matsumoto (91) is only slightly sensitive to UV while all

X-ray sensitive bacterial mutants are also extremely UV-sensitive. It is possible that in yeast there are either two separate mechanisms for modifying UV and X-ray damage or that there is one mechanism with separate branches for handling UV and X-ray lesions. Any model for repair must take into consideration the following observations: 1) X-ray sensitive mutants are only 1.5 to 3 times more sensitive to UV than wild type strains, and 2) an X-ray sensitive allele present in a UV-sensitive strain results in an increase of UV sensitivity by a factor of 2 to 3 (91). These results may indicate that some types of UV damage are only ameliorated by the X-ray repair mechanism or by the X-ray branch of a combined UV-X-ray repair system. One aspect of the present study is to understand more fully the genetic nature of the radiation repair mechanism in yeast.

Though an incongruity between the radiation repair system in yeast and bacteria exists at the level of gene expression, there are significant similarities of these systems in the two organisms. Both have repair systems which are able to modify chemical and UV damage. Snow (129) has reported that UV-sensitive strains of yeast are also sensitive to the inactivating effect of nitrous acid, although the relative sensitivities of the various mutants to this agent are not the same as for UV. Another important similarity in yeast and bacteria is that survival can be increased if the irradiated cells are kept in a non-nutrient medium after irradiation (97, 98). This phenomenon, known as liquid-holding recovery, is intimately associated with dark repair as evidenced by its presence in wild type strains of bacteria and presumably by its absence in UV-sensitive strains (63). It is likely that liquid-holding recovery reflects the increased time

available for DNA repair (52).

The similarities in the repair systems of yeast and bacteria - multiple locus control of radiation sensitivity, ability of a given gene to modify more than one type of lesion, and liquid-holding recovery - support but do not necessarily prove that radiation resistance in yeast is attributable to a repair mechanism equivalent to that in bacteria. However, results obtained in the present study do support the idea that the mechanism of modification of UV damage in yeast is analogous to the repair system found in bacteria. A final proof must come from a biochemical analysis of repair in yeast. Such analysis would be greatly facilitated by the ability to specifically label yeast DNA *in vivo*, a process which at present is not possible since no mutants requiring thymine have been reported.

2. Photoreactivation

Although the presence of a photoreactivating system in yeast has been known since 1952 (138), up to the present its genetic control had not been demonstrated as it had been in bacteria (47). In the present study a photoreactivationless mutant of *S. cerevisiae* has been isolated, and its characteristics examined.

The ability to monomerize UV-induced pyrimidine dimers is a useful tool for studying the problems noted above. The answer to what types of damage are repaired can be obtained in part by utilizing photoreactivation (PR). In bacteria it has been shown that at doses that lead to insignificant inactivation of wild type cells, much of the UV-killing in a sensitive strain can be photoreactivated (122). Based on this evidence and that presented in Section I. B., pyrimidine dimers are a major component of the potentially lethal lesions in

the sensitive strain. In the wild type strain these are efficiently repaired in the "dark". A similar situation is predicted for yeast.

Providing that dark repair is the same in yeast as in bacteria then it should be possible to delimit the step in repair that is altered in a UV-sensitive strain. If no repair occurs, then the same level of survival should be exhibited whether cells are photoreactivated immediately or some time after UV irradiation. However, if some steps in repair are functional, then the enhancement of survival after PR could change with time between UV irradiation and PR. By incubating a UV-sensitive mutant in a non-nutrient medium after irradiation it should also be possible to classify it as "leaky" or "nonleaky": i.e. whether or not some repair activity is present. A "leaky" mutant would be expected to exhibit increased survival with time of incubation because more time would be available for the faulty repair system to function.

Although much can be learned by employing photoreactivation as an investigative tool, an important question as to what causes lethality after maximum photoreactivation still remains. Possibly the remaining lesions are actually dimers which are not susceptible to PR or to dark repair. It is also equally possible that there is non-photoreactivable residual damage of a type that might not be efficiently removed by repair. Other UV-induced lesions (for review see 128) such as a pyrimidine hydrates, inter- or intrastrand cross-links, or strand breaks in close proximity might be types of such non-repairable damage. The nature of the non-photoreactivable sector of lethality remains difficult to assess. However, this sector of damage can be examined with regards to mutation induction.

3. Repair and Mutation Induction in Yeast

A study of mutagenesis has distinct advantages over study of the induction of lethal lesions, because the product of the mutagenic event can be isolated and analyzed.

By examining the production of UV-induced forward and back mutations in UV-sensitive and normal strains, it is possible to assess the nature and effect of UV damage as well as the role of repair in altering the damage. Operationally a forward mutation is detected as a change from wild type phenotype, while a back or reverse mutation returns the mutant phenotype to that of wild type. UV-induced mutagenic lesions can be distinguished in two ways: photoreactivable (pyrimidine dimers) and non-photoreactivable (either pyrimidine dimers or other types of damage). The specific action of these lesions can be determined by examining their ability to revert known mutations and their efficiency in producing various kinds of forward mutations. If the mutations that are reverted or induced by the two classes of lesions are different, then it is unlikely that both classes are due to pyrimidine dimers.

To determine the role of repair in UV mutagenesis, UV-sensitive and normal strains are subjected to equal doses of UV. Differences in mutation induction between the two strains would be attributable to repair. Thus, the role of repair in UV-induced mutagenesis can be evaluated.

In summary the present study investigates the radiation repair systems in yeast and their effect on radiation induced lethality and mutation. It is possible, therefore, to assess the selective advantage of such systems in eukaryotic cells during early evolutionary development.

II. MATERIALS AND METHODS

A. Yeast Strains

Saccharomyces cerevisiae heterothallic strains were used. All strains were obtained or derived from the stocks of Dr. Robert K. Mortimer. Genetic markers are described by conventions adopted at the Yeast Genetics Conference 1961 at Carbondale (136) except that locus and allele numbers (first and second numbers respectively) are printed on the same line as the gene designation (e.g. ar_{4-27} becomes ar 4-27). Lower and upper case letters for a gene symbol indicate mutant and wild type genotypes, respectively.

B. Media

YEPD:

Yeast extract, 1%; Bacto-peptone, 2%; dextrose, 2%; and agar, 2%. (For liquid YEPD the agar was omitted.)

Synthetic Complete (C):

Difco Yeast Nitrogen Base without amino acids, 0.67%; dextrose, 2%; agar, 2%; and the following amino acids: 20 mg/liter adenine (AD), arginine (AR), histidine (HI), methionine (ME), tryptophan (TR), and uracil (UR); 30 mg/liter leucine (LE) and lysine (LY); 100 mg/liter filter-sterilized threonine (THR).

Omission Media:

Synthetic complete minus one or more of the above amino acids; e.g. C-AR.

Special Synthetic Media:

The following were added to synthetic complete in tests of certain strains for the corresponding requirements: 20 mg/liter tyrosine, 20 mg/liter isoleucine, 150 mg/liter valine, 50 mg/liter

phenylalanine, and 60 mg/liter canavanine (in C-AR).

Petite Medium:

Glycerol, 3%; dextrose, 0.025% yeast extract, 1%; Bacto-peptone, 2%; and agar, 2%: used for scoring petite phenotype (inability to utilize glycerol as a carbon source).

Galactose Fermentation Medium:

Yeast extract, 1%; Bacto-peptone, 2%; agar, 2%; and galactose, 2%. The galactose was sterilized separately. Following sterilization these ingredients were mixed and the pH adjusted to 8.0 with $\frac{N}{2}$ NaOH. To this was added 3% (v/v) of a 1% brom-thymol-blue solution in ethanol. Ability to ferment galactose is indicated by change in color of the agar from blue to yellow in the region below the inoculum.

Pre-sporulation Medium (GNA):

Dextrose, 5%; yeast extract, 1%; Bacto-nutrient agar, 2.3%; and agar, 0.5%.

Sporulation media:

The following media were used for sporulation: 1) 3% potassium acetate, 0.002% raffinose, 2% agar (32); and 2) 1% potassium acetate, 0.1% glucose, 0.25% yeast extract, 2% agar (87).

YEPD Discs:

Discs, 1.5 cm in diameter, cut from YEPD plates.

Buffer:

Sorenson's buffer ($Na_2HPO_4 \cdot 2H_2O$, KH_2PO_4), pH 7.

Buffered Agar Discs:

Discs, 1.5 cm in diameter, from buffered agar plates (2% agar; water replaced by the previous buffer).

C. Genetic Analysis

Mating of haploid strains was accomplished by mixing freshly grown cultures of opposite mating types on YEPD. After approximately four hours, zygotes which formed were isolated by micromanipulation.

Sporulation of diploids was promoted by first growing the cells overnight on GNA and then transferring to both types of sporulation media. The sporulation culture was incubated for three days and then examined for the presence of asci. Sporulated samples were then suspended for 60 minutes in a Glusulase (Endo Laboratories, Garden City, New York) solution which had been diluted in water 1:25. Glusulase is an enzyme derived from the crop of snails that digest the ascus wall. After enzyme digestion spore tetrads were separated from each other by microdissection (64).

Random spore analysis was performed by diluting 0.1 ml of the Glusulase treated culture in 10 ml water and sonicating (100 watt Ultrasonic Disintegrator Measuring and Scientific, Ltd.) for 3 minutes at 21 kc/sec (101). Sonication disrupted the spore tetrads and resulted in a suspension that contained mostly single spores, unsporulated diploid cells, and cell debris. This suspension was diluted and plated at a concentration to yield approximately 50 colonies per plate.

Genetic analyses of tetrads for gene-gene linkage and gene-centromere linkage were as described by Mortimer and Hawthorne (88). In a cross AB × ab the following ascus types can be distinguished: parental ditype (PD) AB AB ab ab; non-parental ditype (NPD) Ab Ab aB aB; and tetratype (T) AB Ab aB ab. Gene-gene linkage is indicated by a PD:NPD ratio that is significantly greater than one. The second division segregation frequency of different genes was determined by

examining their segregation relative to known centromere-linked genes (49, 59). Centromere linkage of a gene is indicated by a second division segregation frequency significantly less than 2/3.

D. Radiation Sources

Cells were irradiated with X-rays from a beryllium-window tube (Machlett OEG 60) at a dose rate of 250 r/sec (50 kVp, 25 mA). The UV source was either one or three 8 watt General Electric germicidal lamps (G8T5, 90% of intensity at 2537 Å). The lamps were calibrated with a thermopile (No. 8773, Eppley Laboratory, Inc., Newport, R. I.) which was standardized against a Nelco 50 W, 115 V standard light source. The lamps were periodically monitered with a photocell (No. 935, RCA). The distance between source and surface of irradiation was adjusted to yield a dose rate of 10 ergs/mm^2 (1 lamp) or 60 ergs/mm^2 (3 lamps).

Two sources were used for photoreactivation studies, fluorescent lamps or high intensity blue light. When fluorescent lights (two 15 watt F15T8-WW, General Electric) were used, cells were illuminated for two hours at a distance of 30 cm. High intensity blue light was provided by filtering light from a tungsten floodlamp (3400°C, Sylvania, DWY) with filters (CS 0-51, Corning Glass Works, Corning, N. Y.; and a 4040 Å 2nd order interference filter, Bausch & Lomb). To remove infrared light (132) from the beam, a 15 cm cold water filter was placed between the light source and the filters. Calibration and monitoring of the high intensity blue light source was done by the same procedure used for the UV lamps. The incident intensity of this source was approximately 110 ergs/mm^2/sec; exposure was 20 min. to cells on agar and 30 min. to cells in suspension.

E. Radiation Studies

To induce radiation-sensitive mutations, cells (10^8 cells/ml) were exposed to 0.1 M $NaNO_2$, pH 4.5, for 60 min. (approximately 30% survival) and plated on YEPD after dilution to yield about 50 colonies per plate. The technique of Nakai and Matsumoto (91) was employed to detect the sensitive mutants. The colonies that arose after nitrous acid treatment were replica plated to three YEPD plates. One plate was irradiated with 300 ergs/mm^2 UV, another with 75 kR X-ray, and the third received no irradiation. The plates were examined after one day (UV) or two days (X-ray). Growth of the imprints on the irradiated plates was compared to that on the unirradiated plate. At these exposures, enough cells in the replica imprints had survived to result in a nearly confluent growth. Absence of growth of an imprint on an irradiated plate indicated that cells of the corresponding colony were radiation-sensitive.

Survival curves (Section III. C.) were obtained as follows. Cells of the strain to be studied were inoculated to liquid YEPD (about 10^4 cells/ml) and incubated on a shaker at 30°C for 3 to 6 days. Appropriate dilutions were plated on YEPD plates and the plates were exposed to radiation. The dilution was adjusted to yield approximately 100 colonies per YEPD plate. The irradiated plates were incubated for 3 to 5 days. Survival was determined by comparing the number of colonies on irradiated plates to the number on unirradiated plates.

Two parameters are used to compare irradiation responses with and without PR. The photoreactivable fraction [not to be confused with photoreactivable sector (28)] is defined as that fraction of killing attributed to photoreactivable damage:

$$\text{Photoreactivable fraction} = \frac{S_{PR} - S_{NP}}{100 - S_{NP}}$$

where S_{PR} equals the percent survival after PR and S_{NP} the percent survival without PR. The dose modifying factor, DMF, is the factor of increase in dose required to produce the same survival levels with as compared to without PR. This parameter is also used to compare responses to radiation between strains, provided a constant or nearly constant DMF can be estimated.

The UV-induced reversion study (Section VI) required large numbers of cells since some nutritional requirements reverted at a low frequency (about 5 revertants/10^8 survivors). It was, therefore, necessary to irradiate cells in concentrated suspensions. Cells used for irradiation were obtained from liquid YEPD cultures (about 10^4 cells/ml initial titer) that were incubated at 30°C on a shaker for 4 to 6 days. These cultures were washed twice and resuspended in buffer at about 2×10^8 cells/ml. From this suspension 50 ml aliquots were removed to petri dishes (8.5 cm diameter) and irradiated with UV. The average intensity in the suspension was much less than the incident intensity because of shielding of cells by other cells in the suspension. Therefore, during irradiation the suspensions were mixed with a magnetic stirrer so that all cells would receive the same dose. Since a constant DMF was observed for cells irradiated as a single-layer on agar when compared to irradiation in suspension, all cells irradiated in suspension were considered to have received the same dose. Following irradiation of UV-sensitive strains, with or without PR, the cells were concentrated by centrifugation to a titer of 3 to 5×10^9 cells/ml. Cells at this concentration or after dilution

were then plated on omission media. The number of viable cells on the omission plates was estimated by diluting further and plating on YEPD. The appropriate dilution factor and the number of colonies on the YEPD plates enables a determination of the viable cells plated on the various omission media. A similar procedure was followed for irradiation of wild type cells except that it was not necessary to concentrate the cells since the UV-induced reversion rates were higher. Parallel control experiments (no UV) to determine spontaneous reversion frequencies were also performed.

To test liquid holding recovery of UV-induced damage (97, 98; see Section III. D.) the above irradiated cell suspensions were placed on the shaker in aluminum-covered (no visible light) containers. On subsequent days these cells were subjected to the same procedures that were used immediately after irradiation. All UV-induced reversion and forward mutation studies were performed in a 30° room illuminated by red light.

III. RESULTS: MUTANTS SENSITIVE TO UV AND X-RAYS

A. Isolation of Mutants

Eleven radiation-sensitive mutants were isolated from a sample of approximately 3300 colonies that had arisen after treatement of cells of X1687-101B (a ad 2-1 ar 4-17 ly 1-1 le 1-12 hi 5-2) with nitrous acid. Three of the mutants were UV-sensitive (uvs), seven were X-ray sensitive (xs) and one was sensitive to both types of radiation (uxs).

B. Genetics of Mutants

1. Genetic Properties of Mutants

To assess the genetic nature of these mutants, they were crossed

with non-sensitive strains, sporulated, and the spore clones were analyzed. The analyses of asci from these crosses and the characteristics of the radiation-sensitive mutants are displayed in Table 1. Three of the X-ray sensitive mutants - KC378, KC380, and KC382 - when crossed to non-sensitive strains, exhibited either poor sporulation or low spore viability and were excluded from further study. For the rest of the mutants, 2:2 segregation of the sensitive : non-sensitive phenotype was observed. Thus, the radiation-sensitive phenotypes exhibited by the mutants were concluded to be under the control of chromosomal genes. Since the uxs phenotype also segregated in a 2:2 fashion, it was considered to be due to a single gene mutation rather than mutations in two separate genes, one controlling X-ray sensitivity and the other UV sensitivity. Another mutant with similar properties has been reported by Laskowski, Lockmann, Jennsen and Fink (73).

None of the mutants was linked to any of the genes tested. However the uvs mutants appeared to be centromere-linked since the second division segregation frequencies of these mutants was less than 2/3 (88). The uxs mutant was not centromere-linked based on the frequency of second division segregation resulting from various crosses with this gene (26/44; this study and Fogel, personal communication). Further genetic analyses to determine the centromeres to which the uvs genes are linked are included in Section III. B. 3.

2. Allelism of Mutants

The radiation-sensitivities of the diploids formed by all pairwise crosses of the mutants were examined to determine if any two mutants were at the same locus (allelic). Individual zygotes resulting from

TABLE 1

Numbers of PD, NPD and T Asci* for Radiation-sensitive Vs. Miscellaneous Genes

Mutant Strain	Sensitivity	Numbers of Asci																		Segregation	
		ar 4-17**			hi 5-2			ly 1-1			le 1-12**			thr 1			α			1st	2nd
		PD	NPD	T	PD	NPD	T	PD	NPD	T	PD	NPD	T	PD	NPD	T	PD	NPD	T	Division	
KC370	uvs	3	0	3	2	0	4	1	0	5	1	3	2	1	2	3	2	1	5	4	2
KC371	uvs	1	3	4	0	1	8	4	1	4	3	4	2	1	2	6	1	0	8	6	2
KC373	uvs	3	2	3	2	0	6	1	1	6	2	2	4	2	0	6	3	1	4	5	3
KC372	uxs	3	3	4	3	4	2	1	4	5	4	2	4	3	2	5	0	6	4	6	4
KC376	xs	0	1	6	2	0	7	2	1	4	1	1	6	1	1	5	0	2	4	2	5
KC377	xs	2	2	8	2	1	9	1	5	6	1	4	7	2	1	9	2	4	5	5	8
KC381	xs	3	1	12	3	5	9	6	1	9	1	1	15	2	2	12	0	2	16	2	15
KC383	xs	1	1	5	1	1	6	1	2	4	2	1	5	1	0	7	---			3	5
KC378	xs	No sporulation or low spore viability																			
KC380	xs	"												"							
KC382	xs	"												"							

*PD = parental ditype; NPD = nonparental ditype; T = tetratype (see section II. C.).

**ar 4 and le 1 exhibit, respectively, 16.8% and 4.9% second division segregation (88).

the mating of pairs of mutants were isolated. Streaks of the diploid colonies arising from these zygotes were replica-plated to YEPD and irradiated with UV and X-rays in the same manner used for the isolation and characterization of the radiation-sensitive mutants.

Replica-imprints of diploids homozygous for the mutations (homoallelic) exhibited no growth when irradiated with the dose and type of radiation to which the given mutant was sensitive. However, considerable growth was observed on replica-imprints of mutant X non-sensitive strains; thus, all the mutants were considered to be recessive. Mutants reported by Nakai and Matsumoto (91) and by Snow (129) also were recessive. Allelism, therefore, can be detected as a lack of growth of the irradiated imprints of $mutant_x \times mutant_y$.

Results of the allelism tests are presented in Table 2. The mutations are identified by the numbers of the strains in which they were isolated (Table 1). Only two pairs of mutants isolated in this preliminary study were observed to be allelic: 370-371 and 377-383. Included in this study was $\underline{UV}_1^s$, a UV-sensitive mutant isolated by Nakai and Matsumoto (91). Mutant 373 was allelic with this mutant. Allelism tests of 371 and 373 with a centromere-linked <u>uvs</u> mutant isolated by Snow (129), <u>uvr-9</u>, were also performed; 371 and <u>uvr-9</u> were found to be allelic.

Although it was possible to identify some mutants that were allelic by these tests, other pairs of mutants that may have been allelic could have escaped detection. Such would be the case if two mutants at the same locus were able to complement so that a diploid formed from them was less sensitive to radiation. To resolve whether mutant pairs that yielded non-sensitive responses in the above tests ("+" in

TABLE 2

Responses of Pairwise Crosses of Radiation Sensitive Mutants to UV and X-ray Irradiation

a \ α	uvs (370)	uvs (371)	uxs (372)	uvs (373)	xs (376)	xs (377)	xs (381)	xs (383)
uvs (370)	- / +*							
uvs (371)	- / +	- / +						
uxs (372)	+ / +	+ / +	- / -					
uvs (373)	+ / +	+ / +	+ / +	- / +				
xs (376)	+ / +	+ / +	+ / +	+ / +	+ / -			
xs (377)	+ / +	+ / +	+ / +	+ / +	+ / +	+ / -		
xs (381)	+ / +	+ / +	+ / +	+ / +	+ / +	+ / +	+ / -	
xs (383)	+ / +	+ / +	+ / +	+ / +	+ / +	+ / -	+ / +	+ / -
UV_1^s**	+ / +	+ / +	+ / +	- / +	+ / +	+ / +	+ / +	+ / +
X1687-101B (wild type)	+ / +	+ / +	+ / +	+ / +	+ / +	+ / +	+ / +	+ / +

*Response to radiation (UV/X-ray). A "+" indicates growth of diploid replica-imprint after irradiation, while a "-" indicates no growth.

**Nakai and Matsumoto (91)

Table 2) were allelic or not, the spores of asci derived from these crosses were analyzed. In a small number of asci the identification of at least one non-sensitive spore was considered as sufficient evidence for a mutant pair not being allelic. Tetrad analyses of the pairwise mutant crosses that sporulated are presented in Table 3. Lack of sporulation or poor spore viability observed in some of the crosses may be related to the mutations which cause X-ray sensitivity (see Section III. B. 5.). No further cases of allelism were observed beyond those noted above. Because the uvs mutants were centromere-linked and the xs mutants were not (Table 1), mutant pairs between the two groups were probably not allelic and were not tested.

Based on the previous tests for allelism, there are at least five and possibly six genes described in this study that are associated with radiation sensitivity: two uvs, one uxs, and two or three xs genes. It was not possible to analyze genetically whether 376 was allelic to 381 because of lack of sporulation of the diploid formed from these mutants. However, since the X-ray sensitivities of these two mutants were quite different (see Section III. C. 3.) both as haploid and homoallelic diploids, and the diploid cross of these mutants was not X-ray sensitive, they are considered to be non-allelic. It is conceivable that these mutants are in the same gene and are able to complement each other so that when crossed the diploid is not sensitive to X-rays. The disparity in responses to X-rays could be explained by one of the two mutants being leaky.

The results of the allelism tests are summarized in Table 4. Included in this table are the genetic and radiation characteristics of the mutants as well as the gene and allele assignments given to

TABLE 3 Numbers of PD, NPD, and T Asci

From Crosses of Radiation Sensitive Strains†

Type of Cross	Parents a	Parents α	Percent Sporulation	Number of Asci PD	Number of Asci PD** or T	Number of Asci NPD** or T
xs X xs	376	376	< 1*	-	-	-
"	"	377	0	-	-	-
"	"	381	0	-	-	-
"	"	383	> 1	0	1	5
"	377	377	> 1	7	0	0
"	"	381	> 1	0	1	6
"	"	383	> 1*	-	-	-
"	381	381	0	-	-	-
"	383	383	> 1	3	0	0
uxs X uxs	372	376	> 1	0	0	7
"	"	377	> 1	0	1	5
"	"	381	> 1			
uvs X uvs	371	371	> 1	8	0	0
"	"	373	> 1	0	1	6

†PD (parental ditype); NPD (nonparental ditype); T (tetratype).

*Indicates no spore viability.

**Due to poor spore viability, some asci could only be classified as "PD or T" or "NPD or T".

TABLE 4

Radiation Sensitive Mutants

Mutant	Sensitive to	2nd Division Segregation Frequency †	Mutant gene Designation
uvs (370)	UV	.46	uvs 9-2*
uvs (371)	UV		uvs 9-3*
uvs (373)	UV	.39	uvs 1-2
uxs (372)	UV and X-ray	.59	uxs 1
xs (376)	X-ray	.71	xs 1
xs (377)	X-ray	.62	xs 2-1
xs (381)	X-ray	.87	xs 3
xs (383)	X-ray	.63	xs 2-2

*Allelic to uvr-9 (129).

**Allelic to UV_1^s (91).

† Compiled from data presented in Table 1 and text.

them based on the previously described tests.

3. Centromere Linkage of uvs 9 and uvs 1

To identify the chromosomes on which uvs 9 and uvs 1 (Table 4) were located, strains marked by these genes were crossed to strains that had centromere-linked genes on chromosomes I through XVI. All crosses, except the one involving S_3 (51) a super-suppressor (= S_1 (37)) were analyzed by tetrad analysis. If two centromere-linked genes are on different chromosomes, the expected frequency of ascus types (PD: NPD: T) is 1:1 < 4; on the other hand if they are linked, a few NPD and a much reduced frequency of tetratype (T) asci are expected. A genetic map containing all the centromere-linked genes used in these experiments, except S_3 and ty 7 (121), has been published by Mortimer and Hawthorne (88).

The technique used by Hawthorne and Mortimer (51) for determining the linkage of pairs of suppressors was used to assess whether S_3 was linked to either of the uvs genes. A strain containing the uvs gene to be tested was mated with another that carried S_3 and $cana_1^r$. The $cana_1^r$ allele is suppressible and confers resistance to canavanine. In the presence of S_3 a $cana_1^r$ strain is no longer canavanine resistant. If a sonicated suspension of a sporulated diploid whose genotype is +/uvs S_3/+ $cana_1^r/cana_1^s$ is plated on C-AR+CAN medium, only $cana_1^r$ spores that do not contain S_3 will grow. If a uvs gene is not linked to S_3, then 50% of the spore colonies which arise (all of them "+" regarding S_3) should be UV-sensitive. Neither uvs 9 or uvs 1 was found to be linked to S_3. The frequency of colonies on C-AR+CAN that were uvs was not significantly different from .50 for crosses involving uvs 9-3 or uvs 1-2: 50.9 (251/493) and 52.9 (111/210),

respectively. The results of tetrad analysis of crosses involving either uvs 9 or uvs 1 and the rest of the centromere-linked genes are presented in Table 5. Since the ratio of PD:NPD asci is significantly greater than 1 (16:3) for the gene pair uvs 1 - ty 7, the uvs 9 gene is concluded to be linked to the centromere of chromosome XVI. Based on the evidence which follows uvs 1 is 19.5 centimorgans from the centromere of chromosome XVI and on the arm opposite to that morked by ty 7. 1) The second division segregation frequencies (SDS) of uvs 1 and ty 7 are .32 (12/37) and .54 (22/41), respectively. When results for all experiments in the present study are included, the SDS for uvs 1 is .39 (37/94). If uvs 1 and ty 7 are on the same arm of chromosome XVI, no NPD are expected contrary to observation. 2) Among the 19 tetratype asci 5 were second division for uvs 1, 13 were 2nd division for ty 7, and 1 was 2nd division for both markers. These results would not be expected if ty 7 were linked to uvs 1 on the same arm of chromosome XVI.

The uvs 9 gene appears to be unlinked to any of the centromere-linked genes identifying chromosomes I through XVI in Table 4. It is considered to mark the centromere of chromosome XVII (previously unidentified genetically) at a distance of 23 centimorgans (SDS is .46, 22/48). These results bring the number of chromosomes based on genetic evidence to one less than the 18 observed cytologically by Tamaki (134).

4. Suppressibility of Mutants

Markovitz and Baker have reported the suppressibility of radiation sensitivity* by an ochre suppressor, and concluded that the product of the corresponding gene is a polypeptide (86). Similarly the

*In bacteria.

TABLE 5

Numbers of PD, NPD, and T* Asci for uvs 9-3 and uvs 1

Vs. Centromere-linked Genes

Chromosome number	Centromere-linked gene	uvs 9-3			uvs 1-2		
		PD	NPD	T	PD	NPD	T
1	ad 1	1	2	4	3	4	7
2	ga 1	1	2	6	3	3	6
3	hi 4	2	1	19	3	5	12
4	tr 1	3	1	9	4	3	5
5	ur 3	3	3	8	3	5	4
6	hi 2	3	1	7	1	2	9
7	le 1	3	4	2	3	1	4
8	ar 4	1	3	4	3	2	3
9	ly 1	7	4	17	1	1	6
10	is 3	10	3	11	5	3	3
11	met 14	4	2	4	9	4	10
12	thr 5	4	2	4	3	1	9
13	ly 7	3	3	8	1	1	10
14	p 8	4	2	5	3	4	4
15	S_3**	-	-	-	-	-	-
16	ty 7	3	2	10	16	3	19

*PD = parental ditype; NPD = nonparental ditype; T = tetratype. Summary of results from various crosses.

**See text.

suppressibility of the radiation-sensitive mutants in the present study was tested. Revertants arising on replica-imprints of the mutants on C-LY-HI were isolated. Since these revertants were presumably due to the presence of super-suppressors (see Section VI. B.), the suppressibility of the radiation sensitive mutants could be tested by determining the radiation sensitivity of the revertant isolates. None of the radiation-sensitive mutants was resistant when a super-suppressor for ly 1-1 and hi 5-2 was present. Some of the mutants, however, may still be suppressible since all possible suppressors are not selected by this system (37, 51).

5. Sporulation of Radiation Sensitive Mutants

All pairwise crosses of the uvs and uxs mutants exhibited good sporulation and spore viability. However, either no sporulation or no viable spores were observed in the crosses xs 1/xs 1, xs 3/xs 3, or xs 1/ ± xs 3/ ±. The xs 1 and xs 3 genes, therefore, seem to affect the sporulation process. Another X-ray sensitive mutant of yeast, reported by Puglisi (100), also prevents sporulation. This mutant differs from xs 1 and xs 3 in that it is dominant and has not been isolated in a haploid. It is possible that mutants of these genes interfere with recombination during meiosis in a manner analagous to that reported in recombinationless mutants of E. coli (14, 58). Similarly, UV-sensitive mutants of Neurospora crassa (72), Aspergillus nidulans (13) and Ustilago maydis (55) that affect meiosis have been reported.

Lack of sporulation associated with xs 1 and xs 3 is a recessive trait since sporulation was observed in crosses involving these genes with other xs strains. The inability of the cross heterozygous for

both xs 1 anc xs 3 to sporulate may be due to these genes being allelic (see Section III. B. 2.). On the other hand, factors not associated with these mutants may prevent sporulation. No sporulation was observed in a cross involving xs 1 and xs 2-1, while in the cross xs 1 × xs 2-2 good sporulation and spore viability occurred.

C. Sensitivity of Mutants to X-rays and UV

1. uvs Mutants

Haploid and homozygous diploid strains marked by any one of the mutations uvs 9-2, 9-3, or uvs 1-2 are very sensitive to UV irradiation (Fig. 1 and 2). The dose modifying factor, DMF, of these strains compared to the wild type parent-strain (X1678-101B) and a wild type diploid was between 20 and 30 in the dose range examined. Although the mutants were sensitive to UV, no increased sensitivity to X-rays was observed (Fig. 3). The responses of these mutants to UV and ionizing radiation are therefore in agreement with those reported for uvr-9 (129) and UV_1^s (91) to which uvs 9-2, 9-3 and uvs 1-2, respectively, are allelic.

UV survival curves of the uvs mutants are also presented in Fig. 4 (the dose scale is expanded from that in Fig. 1 and 2). All the curves have a shoulder at low doses, indicating that a low level of repair may occur in the uvs mutants or that a threshold accumulation of UV damage is required for the killing of a cell. Another type of repair, i.e. photoreactivation (for a discussion of photoreactivation see Section IV. A.) resulted in the same level of survival for the three uvs strains (Fig. 4). Since photoreactivation removes pyrimidine dimers (115) and at the doses applied to the uvs strains the survival of the wild type haploid was nearly 100% (without PR), the dark

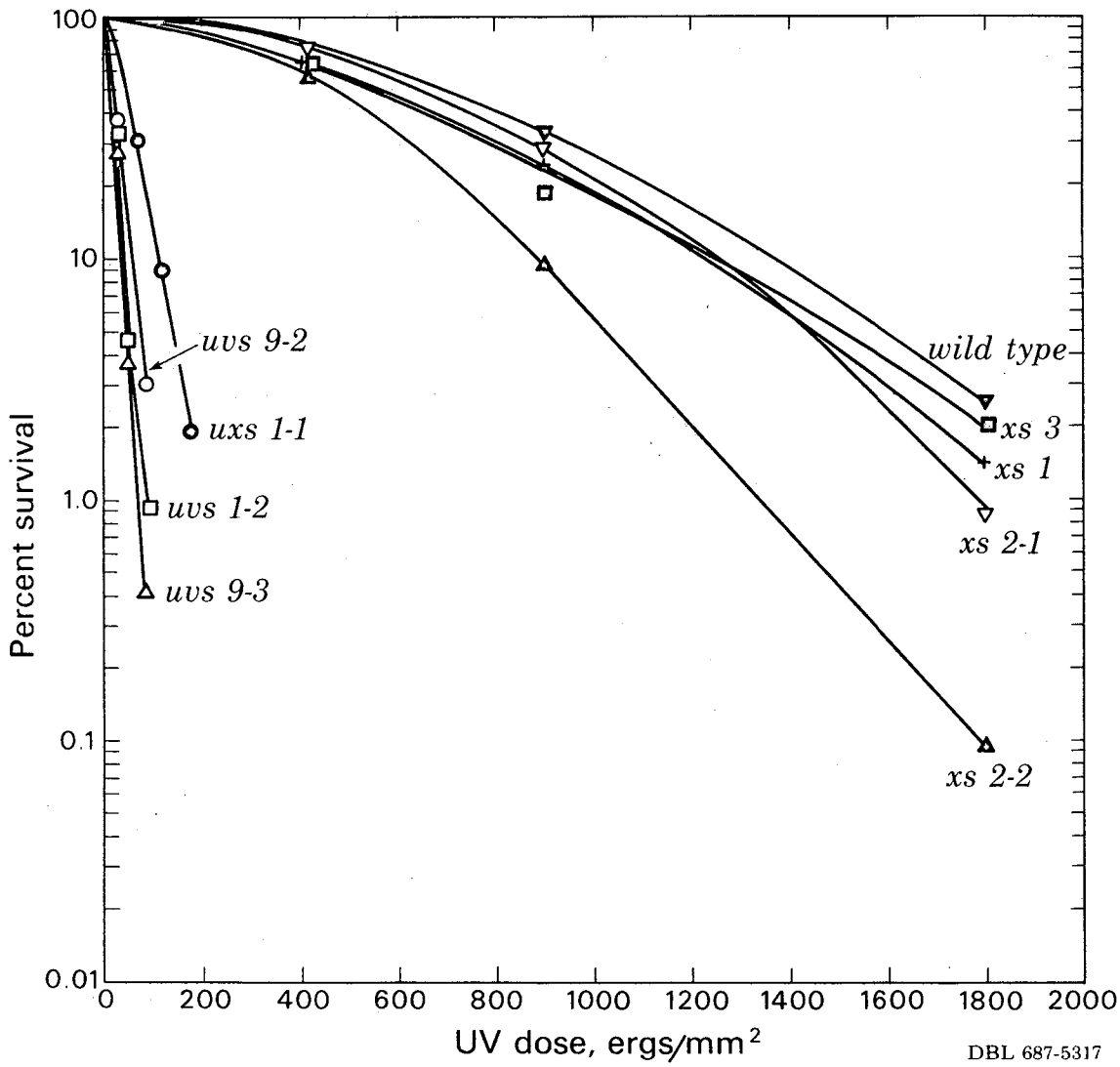


Figure 1. Survival after UV-irradiation of haploid radiation-sensitive strains.

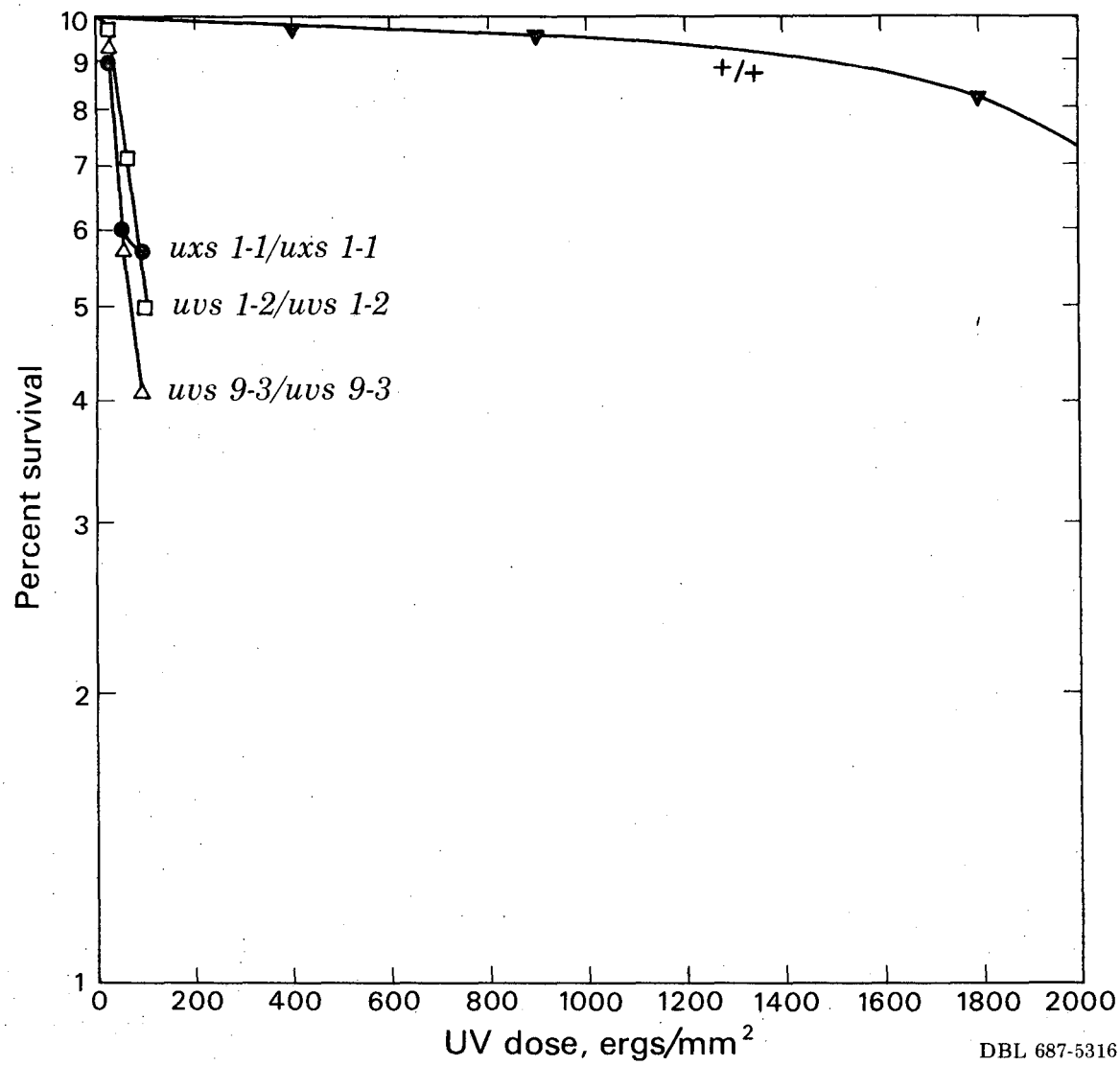


Figure 2. Survival after UV-irradiation of diploid strains sensitive to UV.

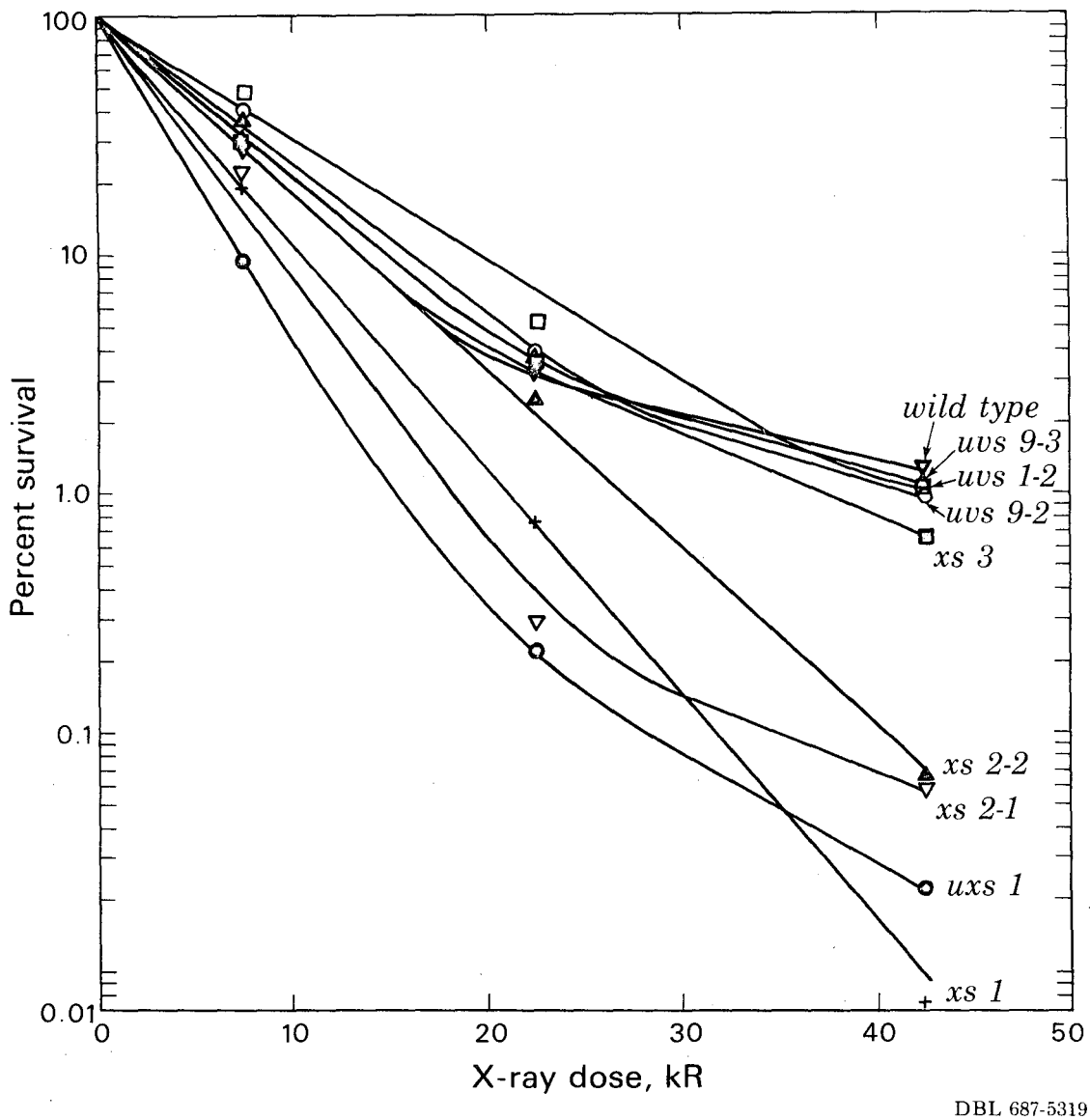


Figure 3. Survival after X-ray irradiation of haploid radiation-sensitive strains.

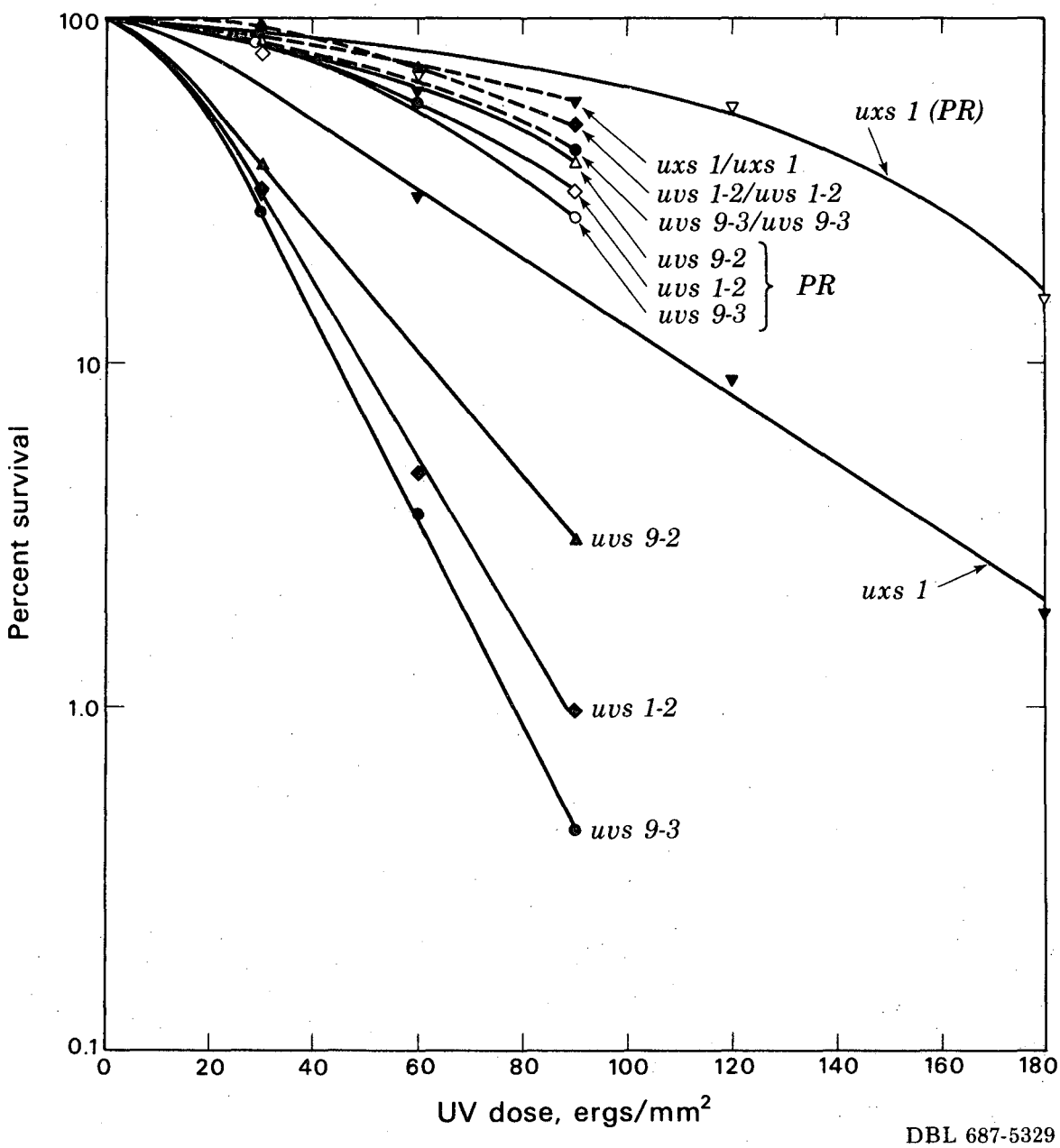


Figure 4. Survival after UV-irradiation of UV-sensitive haploid and diploid strains. Closed and open symbols: No PR and PR, respectively. Solid and dashed lines represent haploid and diploid responses, respectively.

repair system in yeast is very efficient in removing pyrimidine dimers. This repair system in yeast is also efficient in removing the damage that remains following PR in the uvs strains; this sector of damage may be due to pyrimidine dimers remaining after PR or other lesions.

2. uxs Mutant

Radiation sensitivity of the uxs mutant is similar in some respects to that of the mutant r_1^s reported by Laskowski, et al. (73), in that both are sensitive to UV and X-irradiation. The uxs haploid and homozygous diploid strains are 1.9 and 3.0 times more sensitive to X-rays, respectively compared to a wild type haploid and diploid strain (Fig. 3 and 5). For the r_1^s mutant corresponding values are about 2 and 2.5. With regard to UV sensitivity, the uxs 1 mutant was almost as sensitive as the uvs 1 and uvs 9 mutants (Fig. 1). The r_1^s mutant was different in that it was much more resistant than uxs 1 to UV. The DMF's were between 10 to 16 (Fig. 1 and 2) for both haploid and diploid uxs mutants, whereas the corresponding values were 3 to 6 and 2.6 for r_1^s. Such large differences in the UV sensitivity of uxs 1 and r_1^s indicates that these are mutants of two different genes. If, instead, they were mutants at the same locus then the differences in UV sensitivity might be attributed to a "leakiness" of the r_1^s mutant. Such leakiness might be expected to affect repair of X-ray as well as UV-induced damage. Since the X-ray sensitivies were comparable, it is unlikely that the r_1^s mutant is a leaky mutant of the uxs 1 locus, but is instead a mutant of a different gene.

3. xs Mutants

At low doses, the X-ray responses of the xs mutants were similar

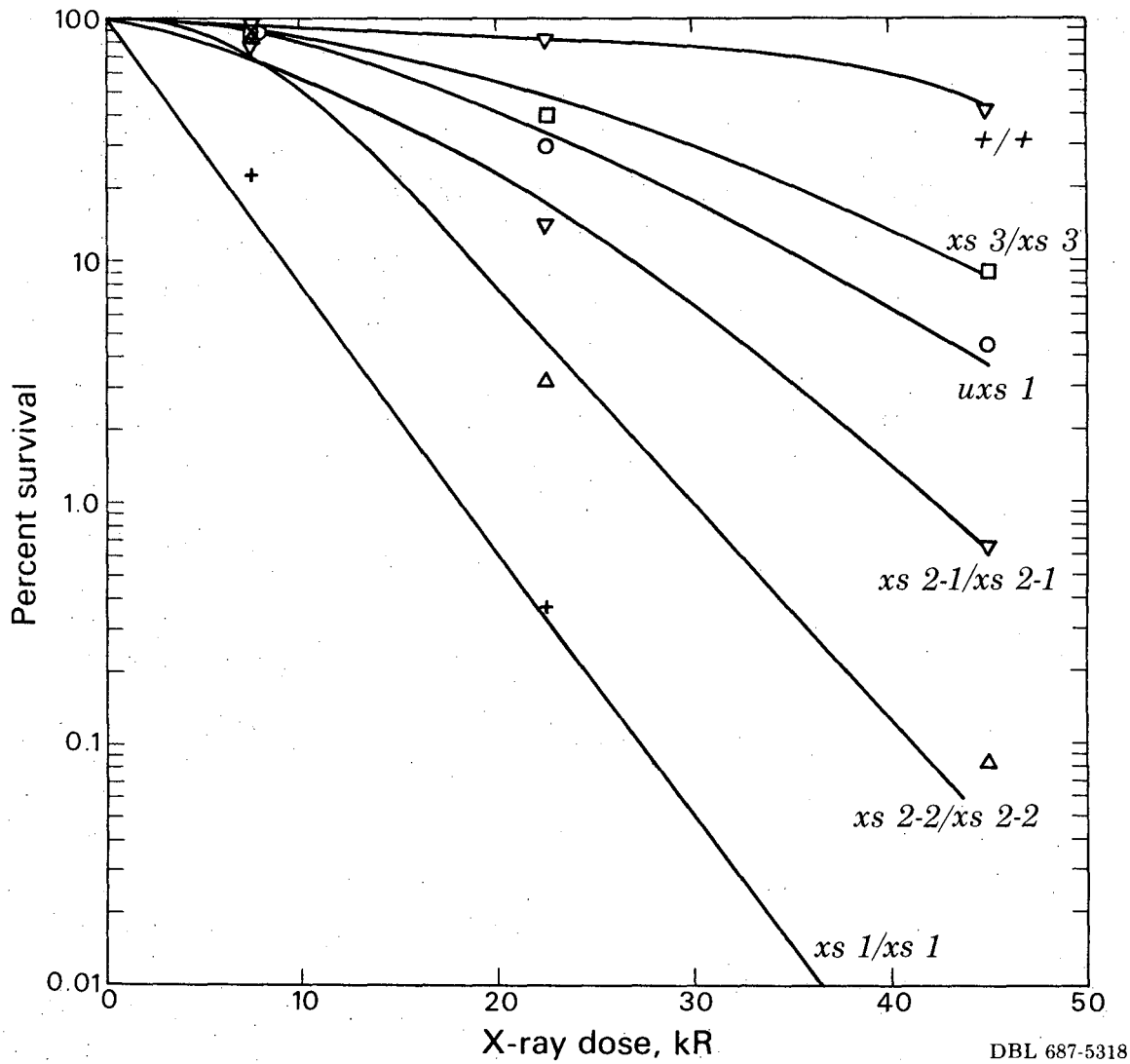


Figure 5. Survival after X-ray irradiation of diploid radiation-sensitive mutants.

to that of the wild type strain (Fig. 3). The DMF's of these mutants were between 1 and 1.5. However, at high doses the "tail" that was present on the X-ray survival curve of the wild type strain was either absent or much lower for the xs mutants. The presence of a resistant "tail" has been attributed to budded cells in the irradiated population (2). These budding cells are much more resistant to X-rays than interdivisional cells. Since the frequency of budded cells was about 10-20% for the irradiated xs and XS cells, the xs phenotypes result from alterations of genes that normally confer resistance to X-rays during budding.

The decrease of the shoulders in the X-ray survival curves of the xs/xs diploid strains when compared to the wild type XS/XS (Fig.5), indicated that the genes controlling X-ray resistance during the haploid budding stage also affect the resistance of diploid cells. The wild type diploid was at least 2 times more resistant to X-rays compared to any of the diploids homozygous for xs mutations (Fig. 5). Furthermore, the amount of reduction of the shoulder in the survival curve of xs/xs strains was found to be related to the extent of lowering of the "tail" of the haploid survival curve. The xs 2 mutants exhibited lower "tails" compared to xs 3. Similarly xs 2/xs 2 diploids had smaller shoulders than the xs 3/xs 3 diploid. Based on these results, the survival curve of the xs 1/xs 1 diploid was not expected to exhibit a shoulder since no "tail" was observed for the haploid survival curve. The results are in agreement with this prediction. The correlation between the shoulder and the "tail" in the survival curves of xs/xs diploids and xs haploid strains, respectively, indicates that the factor that confers resistance to X-rays in budding haploid cells

also confers resistance to diploid cells. These observations may result from an alteration of an X-ray repair mechanism that is activated during the budding cycle of haploids, but which is active in all division stages of diploids. This mechanism of repair, however, requires further investigation.

All of the xs mutants, except xs 2-2, had the same sensitivity to UV as the wild type (Fig. 1). Since the strain carrying the xs 2-2 mutation was about 1.5 times as sensitive to UV as a strain with xs 2-1, which was not sensitive to UV, xs 2-1 is probably a leaky mutation of the xs 2 gene. This is corroborated by the increased X-ray resistance of the xs 2-1/xs 2-1 diploid over the xs 2-2/xs 2-2 diploid (Fig. 5). Therefore, the xs 2 locus can be considered to affect X-ray sensitivity and to some extent UV sensitivity, whereas the xs 1 and xs 3 loci affect only sensitivity to ionizing radiation. The xs 1 mutant appears similar to X_1^s reported by Nakai and Matsumato (91) in that the X-ray survival curves of the haploid xs 1 and the diploid xs 1/xs 1 strains are exponential and nearly identical. However, the X_1^s strain is sensitive to UV while strain xs 1 is no more sensitive than the wild type. Thus, they might be mutations of two different loci.

On the basis of their UV and X-ray sensitivities and the genetic analyses of the various xs and uxs mutants in the present study, it is concluded that there are at least four loci that can modify the response of yeast cells to X-rays. Two loci, xs 1 and xs 3 affect only sensitivity to X-rays, and two other loci uxs 1 and xs 2 affect UV sensitivity as well. If the other two xs mutants that have been reported in yeast r_1^s (73) and X_1^s (91), are not allelic to any of the above or to each other (as seems to be the case based on their responses to

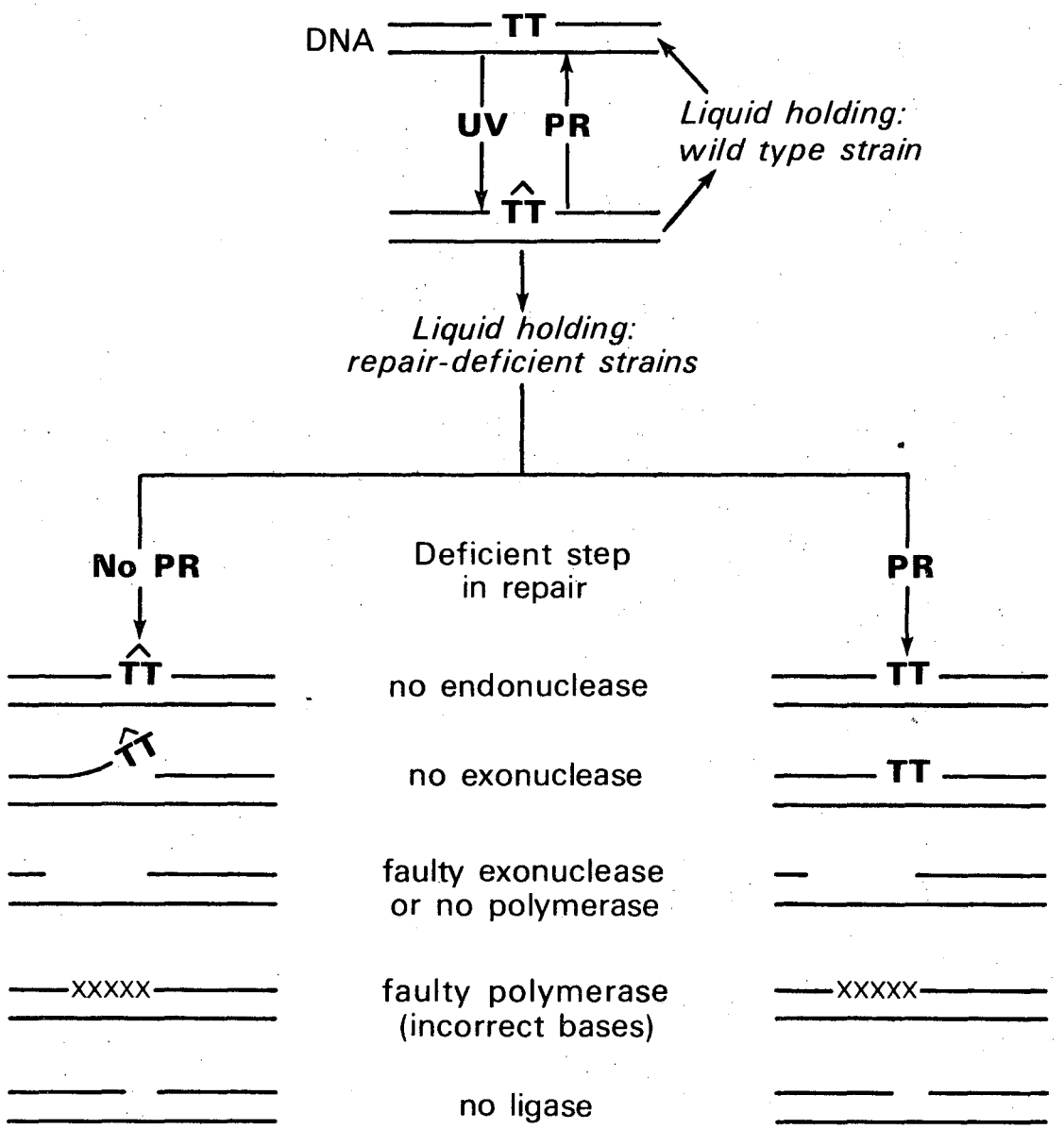
X-rays and UV), then there are at least six loci in yeast that control X-ray sensitivity.

D. Repair Deficiency Associated with uvs 9-3

1. Recovery After Irradiation

As discussed in Section I. C. 2., it should be possible to distinguish between various kinds of "repairless" mutants on the basis of their photoreactivability after UV-irradiation. For example, the PR sector of damage for mutants that are incapable of beginning either incision or excision, the first two steps of repair, would be expected to remain constant with time after UV provided DNA synthesis is prevented. For mutants that have an incorrectly functioning excision enzyme (analogous to a "reckless" mutant of *E. coli* (14, 58) or a defect in later steps of repair the PR sector of damage would probably decrease since excision involves the removal of pyrimidine dimers (Fig. 5a).

To delimit the faulty step in repair the response to UV of KC614-6-24B (*uvs* 9-3) was examined in a manner that would detect whether or not UV-induced dimers can be removed in this strain. Survival of cells irradiated in buffer was examined immediately (Day 0) and on the fifth day (Day 5) after irradiation; during this time they were kept in buffer (see Section II. E.). The photoreactivability of cells at these times was determined by exposing aliquots of the irradiated cell suspension to blue light and measuring survival. If sensitivity results from a lack of functional incision or excision enzyme, the photoreactivable fraction should remain constant irrespective of how long after UV-irradiation cells are photoreactivated. However, the photoreactivable fraction should decrease with time if sensitivity of the strain is due to the presence of an incorrectly



DBL 687-5320

Figure 5a. Proposed alteration of UV-induced pyrimidine dimers in wild type and repair-deficient strains.

functioning excision enzyme or a defective enzyme involved in a later step in repair. It is assumed that the liquid holding process does not prevent dark repair enzymes from acting since in wild type strains these enzymes can function when cells are placed in buffer after UV-irradiation (liquid holding recovery (98)). Survival in the absence of PR would be expected to remain constant or increase slightly depending on the degree to which the incision or excision enzyme was altered.

Shown in Table 6 are the results of experiments in which the survival, with and without photoreactivation, of UV-irradiated cells was measured on Day 0 and Day 5. The average percent survival in the absence of PR increased from 1.0% to 2.6%. But the average survival after photoreactivation on either Day 0 or Day 5 was approximately the same (36% vs. 41%). Therefore, the amount of damage attributable to pyrimidine dimers remained constant during the five days following irradiation. Based on the criteria presented earlier in this section, the uvs 9 gene probably controls a step in repair involving either incision or excision. The increase in survival over a period of 5 days may have been due to leakiness of the particular mutant involved. The shoulder in the survival curve of this mutant (Fig. 4) indicates further that the uvs 9 mutant is leaky.

2. Photoreactivation After Incubation in YEPD

Photoreactivability of cells held in buffer was also measured as a function of time after resuspension in liquid YEPD. If the presence of pyrimidine dimers blocks DNA synthesis (125), then photoreactivation at various times after resuspension of the irradiated cells should result in constant levels of survival since PR removes the barriers to DNA synthesis. However, if the photoreactivable fraction decreases

TABLE 6

UV survival immediately following irradiation and after 5 days in buffer of the UV sensitive strain KC614-6-24B (uvs 9-3)

Exp.	Days in Buffer	% Survival No PR	PR
14	0	1.0	26.6
18	0	0.53	33.4
27	0	1.4	49.3
14	5	4.2	41.2
18	5	2.2	38.9
27	5	1.3	41.9

gaps in newly synthesized DNA. These gaps or breaks if not filled might prevent DNA synthesis in subsequent generations or conceivably they would increase the possibility of chromosome breakage. Photoreactivation would probably not alter the presence of gaps or breaks in newly synthesized DNA since the dimers are in the template DNA. The probability of gaps being produced would increase with time of incubation. Therefore, the possibility of photoreactivation of lethal damage would decrease. On the basis of this hypothesis for lowered photoreactivability with the onset of DNA synthesis, it is expected that "breaks" or gaps are produced during DNA synthesis in S. cerevisiae after UV-irradiation. Also, either the stability of these alterations or an increase in the number of chromosomal breaks should be detectable with increased time of incubation in liquid YEPD.

c) If DNA replication proceeds past the dimer, incorrect bases might be inserted. As a consequence the newly formed strand of DNA would have several altered sites while the template strand would contain several pyrimidine dimers. Photoreactivation would remove the dimers in the old strand but would not affect the mutant sites in the newly synthesized strand. If cell killing results from mutations in genes critical to cellular function (e.g. genes corresponding to ribosonal proteins or polymerases) then the photoreactivable sector should decrease with time of incubation because the dimers would have already exerted their effect by causing pairing errors.

Although pyrimidine dimers may cause killing by the production of mutations in haploids, it is unlikely that lethality in diploids also result from such mutations. Based on results in Sections V. D. 1. and 2., forward mutations produced by pyrimidine dimers are mainly base-pair

substitution events. Assuming that such events are expressed recessively, their contribution to lethality in a diploid would be small unless lesions occurred in the same genes of homologous chromosomes. This possibility seems unlikely.

IV. RESULTS: A "PHOTOREACTIVATIONLESS" MUTANT OF YEAST

A. Introduction

The genetic control of what was assumed to be dark repair in yeast was examined in the previous section. The dark repair probably occurs through a stepwise process similar to that discussed for repair in bacteria (Section I. B.). From the number of loci identified and the different phenotypes of mutants at these loci, one can conclude that more than one enzyme is involved.

Another type of repair of UV lesions - photoreactivation (PR) - is more direct. First described by Kelner (66), it is characterized by an increase in survival of UV-irradiated organisms when they are exposed to light of longer wave length after irradiation. The enzyme which mediates such repair - the photoreactivating enzyme - has been isolated from yeast and its activity examined. Rupert has shown that it attaches directly to UV-inactivated transforming DNA. After PR, the enzyme no longer remains attached and the biological activity of the DNA is returned (102, 104). Furthermore, PR has been shown to reduce *in vivo* the number of UV-induced pyrimidine dimers in both prokaryotes and eukaryotes (125, 133). Other experiments demonstrate that the reduction in the number of pyrimidine dimers after PR is accompanied by a corresponding increase in non-dimerized pyrimidines in the DNA (16, 124).

Thus, the photoreactivating enzyme can be considered to function

by first attaching to a section of DNA that contains the UV-induced pyrimidine dimer to form an enzyme-dimer complex which can then be activated by light of a longer wave length (3600-4200 Å (116)) to cleave the pyrimidine dimer bonds. The pyrimidine bases are thus returned to their original state and the enzyme no longer remains attached. (Photoreactivation and its mechanism are discussed further in reviews by J. K. Setlow (114) and Rupert and Harm (106)).

Although the ability to photoreactivate UV damage has been shown to be present in many diverse organisms (17, 60), there has been little study of the genetic control of photoreactivation. The only "photoreactivationless" mutant (phr⁻) thus far reported has been isolated in Escherichia coli B (47). PR applied after UV irradiation does not enhance survival in the phr⁻ strain, even though the same treatment to the parent strain greatly increases the number of survivors.

The present study describes a mutant isolated from S. cerevisiae that is "photoreactivationless", phr. Since this yeast can be maintained as either a stable haploid or diploid (or at even higher ploidies), it is possible to assess the dominance of the PHR gene and, furthermore, to examine the effect of gene dosage.

B. Procedure

It was considered more convenient to isolate the phr mutant in a uvs strain, rather than a UVS strain. Survival of the wild type strain is much less affected by PR after low doses of UV, when compared with a uvs strain, (Fig. 7). Thus, a phr mutant in a uvs strain would be expected to be more readily discernible than the same mutant in a UVS strain.

The method of isolation of the phr mutant was similar to that

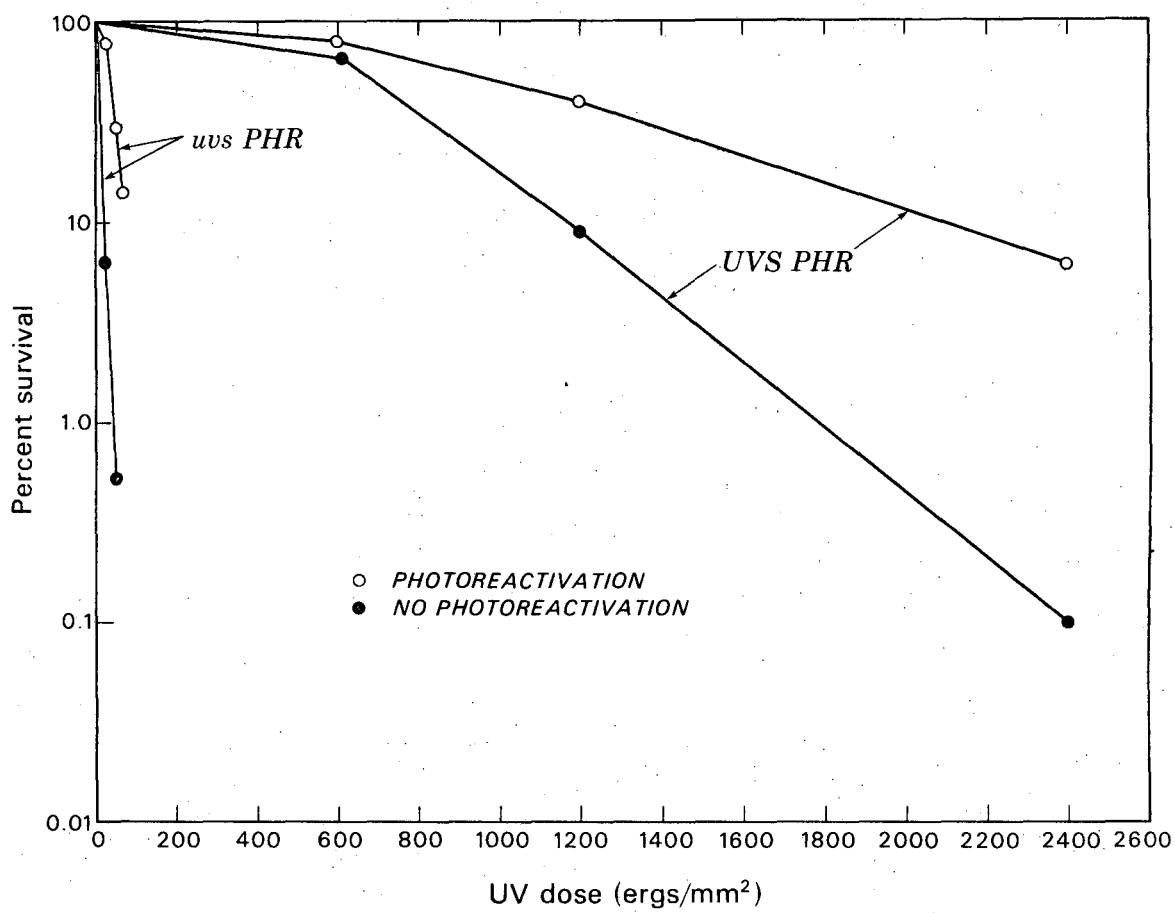


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Figure 7. Survival of a UVS and a uvs strain when UV-irradiation is followed by photoreactivation or no photoreactivation.

used for the isolation of uvs mutants. A four day old YEPD culture of uvs 9-3 (KC614-6-24B: α le 1-12 ar 4-27 ad ly 1-1 thr 4-1 hi 5-2 uvs 9-3) cells was washed, irradiated with UV (about 20% survival) to induce possible phr mutations, and plated to nutrient agar. When colonies developed, each plate was replica-plated to two YEPD plates, both of which were exposed to UV (200 ergs/mm^2). Immediately following exposure to UV, one plate was placed in the dark and the other was illuminated by fluorescent lights for two hours (see Material and Methods) and then placed in the dark. One day later the replica-imprints were examined. A phr uvs 9-3 mutant was expected to have a low level of growth on both plates while the other colonies (PHR uvs 9-3) should exhibit much more growth on the plates exposed to light. Among approximately 20,000 colonies tested, one mutant with the phenotype expected of a phr mutant was isolated.

The effect of the phr mutation was examined in detail in various haploid and diploid strains by determining in more detail its influence on survival after UV-irradiation with and without PR. Survival curves were determined by plating an appropriately diluted four to six day liquid YEPD culture to YEPD plates. These plates were then irradiated (dose rate: 10 or 60 ergs/mm^2/sec, depending on the sensitivity to UV of the strains involved). The procedure for photoreactivation following UV-irradiation was the same as used when searching for phr mutants. Colonies arising after three days were counted and survival curves determined.

C. Results

1. Genetics of the phr Mutant

The phr mutant isolate was crossed to another uvs 9-3 PHR strain

to determine its genetic nature. In tetrads derived from the cross PR10 = PR3 × KC614-6-23C (α le 1-12 ar 4-27 ad ly 1-1 thr 4-1 hi 5-2 uvs 9-3 phr × a le 1-12 ar 4-17 ad 2-1 uvs 9-3) regular 2:2 segregation of the phr:PHR phenotype was observed. Thus, the mutation was considered to be an alteration of a chromosomal locus, phr 1.

Displayed in Table 8 is an analysis of tetrads from PR10 and additional crosses involving PR3. From these results it can be concluded that phr 1 is not linked to any of the following genes: ly 1, hi 5, thr 4, uvs 9-3, ar 4, le 1, ur 1, me 1, or the mating type locus. Furthermore, phr 1 is not centromere-linked; the second-division segregation frequency was 73% (27/37).

2. Influence of phr 1 on UV-induced Lethality

As shown in Fig. 8, UV sensitivities for the four spore isolates from an ascus of PR10 (PHR 1/phr 1 uvs 9-3/uvs 9-3) were comparable as expected because all four spores contained the uvs gene. Since two spores were phr 1 and two were PHR 1, the phr 1 gene can be considered to have no effect on the sensitivity of these isolates to UV in the absence of PR. As anticipated, survival of PHR strains was considerably enhanced when photoreactivating light was applied after UV-irradiation whereas no enhancement was observed for the phr strains.

Similar results were obtained when the effect of the phr 1 gene was examined in UV-resistant haploids and diploids as well as in a uvs homozygous diploid (Table 9). Within different individual pairs os strains, the sensitivities to UV were comparable (Fig. 9). However, the survival of PHR strains was always increased by exposure to photoreactivating light after UV-irradiation, while the survival

TABLE 8

Numbers of PD, NPD, and T Asci
for phr 1 Vs. Various Genes*

Gene	Numbers of Asci		
	PD	NPD	T
a	3	9	25
hi 5	9	8	32
le 1	6	5	27
ly 1	2	1	4
me 1	11	5	23
thr 4	6	9	45
ur 1	6	5	27
uvs 9-3	3	5	8

*PD = parental ditype, NPD = nonparental ditype,

T = tetratype.

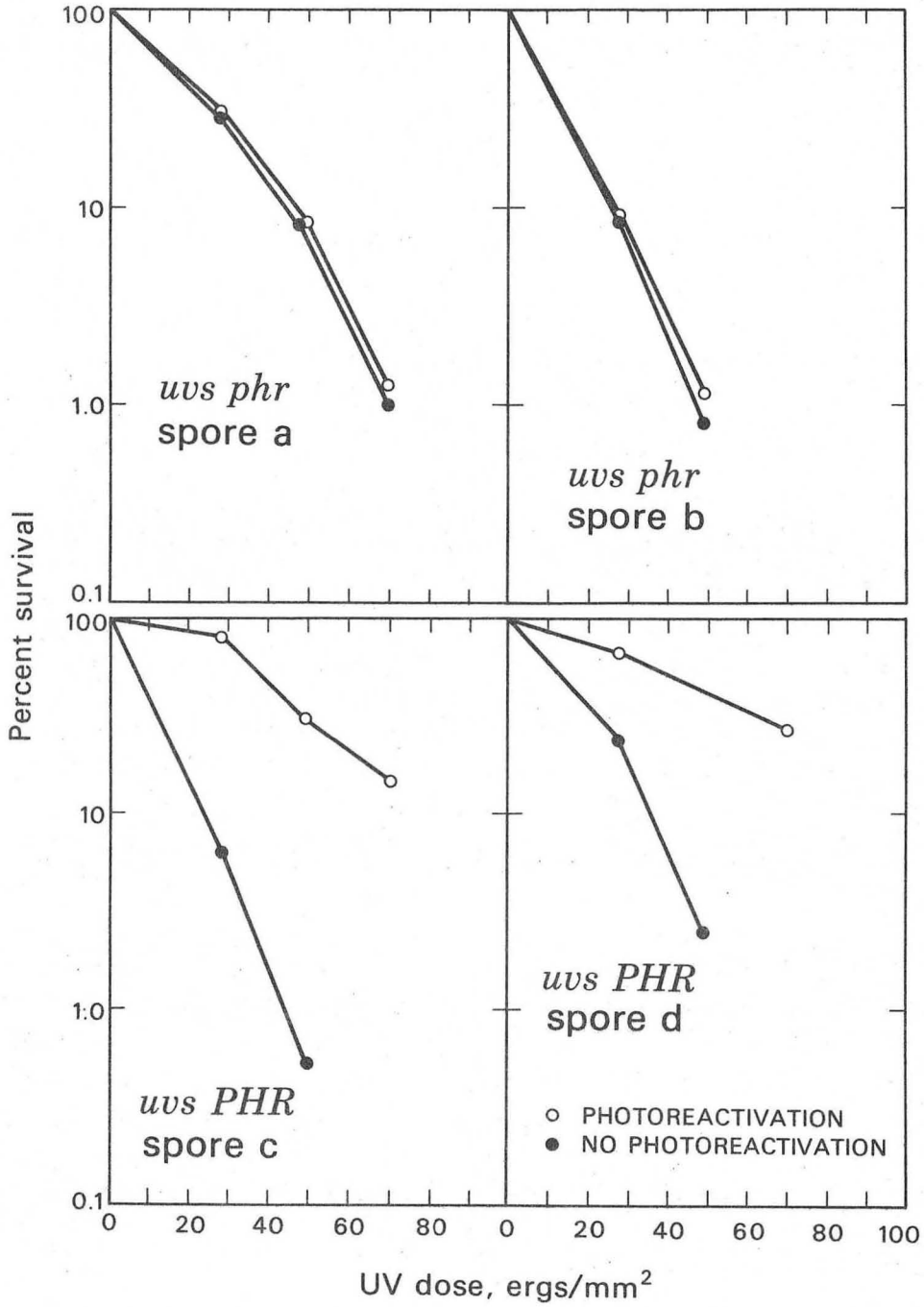

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Figure 8. Survival of the four spore isolates from an ascus of PR10 (PHR 1/ phr 1 uvs 9-3/ uvs 9-3) when UV-irradiation is followed by photoreactivation or no photoreactivation.

TABLE 9

Photoreactivation of Various Strains Marked by phr 1 and PHR 1

Strain	Ploidy	Genotype		Photoreactivation Dose Modifying Factor
PR11-27C	1n	PHR 1	UVS	1.6 - 1.9
PR11-12C	1n	phr 1	UVS	0
PR17	2n	PHR 1/PHR 1	UVS/UVS	1.6 - 1.9
PR16	2n	phr 1/phr 1	UVS/UVS	0
PR13	2n	PHR 1/PHR 1	uvs 9-3/uvs 9-3	2.9 - 3.1
PR12	2n	phr 1/phr 1	uvs 9-3/uvs 9-3	0

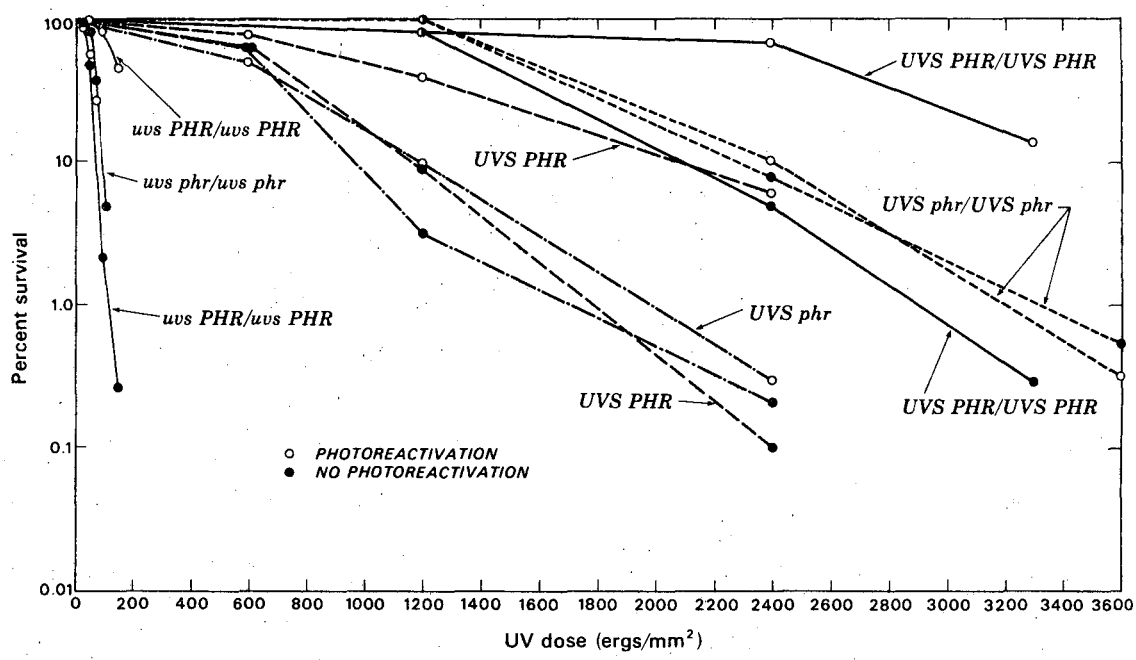

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Figure 9. Survival of UVS and uvs 9-3 haploid and diploid strains that are either capable (PHR) or incapable (phr) of photoreactivating UV-induced lethal damage.

of phr strains was not altered. Thus, it can be concluded that the product of the PHR gene has an effect on survival only when UV irradiation is followed by photoreactivating light, regardless of ploidy or sensitivity to UV.

3. Dominance of PHR Gene

To determine the dominance of the PHR gene, the photoreactivation responses of two diploid strains, one homozygous for PHR 1 (PR13: PHR 1/PHR 1) and the other heterozygous (PR14: PHR 1/phr 1) were measured. These strains were chosen because they were homozygous for UV-sensitivity (uvs 9-3/uvs 9-3). The photoreactivable fraction is larger in uvs than in UVS strains, therefore, differences in response between PHR 1/PHR 1 and PHR 1/phr 1 diploids could be more easily distinguished. Fig. 10 displays the survival curves of strains PR13 and PR14. Although the UV survival curves of these strains are not identical, the effect of photoreactivation can be estimated by measuring the dose modifying effects on survival. The DMF for photoreactivation of the homozygous PHR strain was 2.8 - 3.1, whereas for the heterozygous strain it was 2.9 - 3.3. Since these values are nearly equal, it can be concluded that the photoreactivability remains the same whether the PHR 1 gene is homozygous or heterozygous. Therefore phr 1 is recessive to PHR 1.

It is surprising that the ability to photoreactivate UV damage is nearly equal in the homozygote and the heterozygote. If the synthesis of photoreactivating enzyme is regulated in a manner similar to other gene-enzyme systems in yeast (40, 71, 93, 127) in which specific activity increases linearly with gene dosage, the specific activity of the photoreactivating enzyme in a homozygous strain should be twice

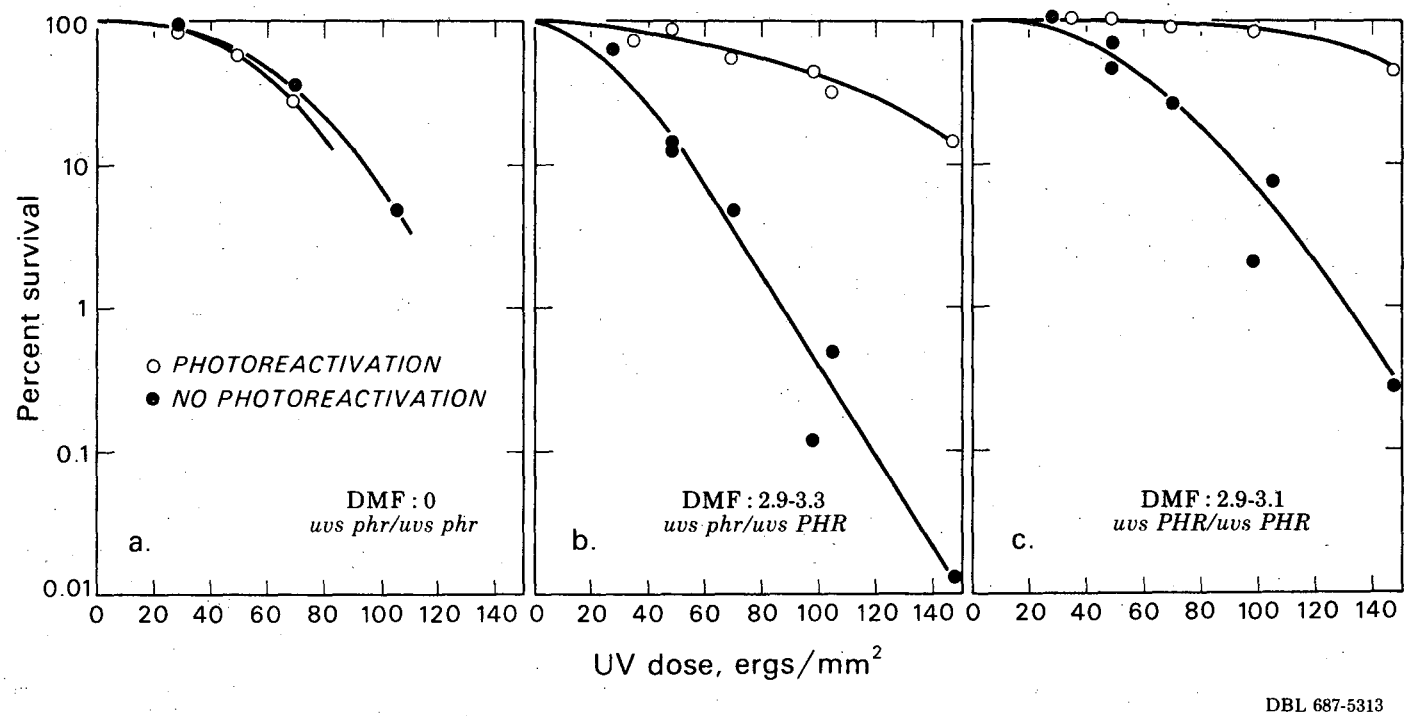

Figure 10. Survival of diploid strains with none, one, and two PHR 1 genes when UV irradiation is followed by photoreactivation or no photoreactivation.

that in a heterozygote. Consequently, a difference in photoreactivability of the two strains might be expected. The lack of a difference between the homozygote and the heterozygote might be attributed to either of the following reasons: 1) A regulatory mechanism may control the amount of enzyme produced independent of the dominant allele. 2) The homozygote could produce twice as much photoreactivating enzyme as the heterozygote but the amount of enzyme exceeds that needed to remove the dimers produced at the UV doses employed. If this is the case, non-photoreactivable lethal damage may be due to lesions other than pyrimidine dimers. To distinguish between the above two possibilities a direct analysis of photoreactivating enzyme specific activity in extracts of the two strains is necessary.

Evidence presented by Boling and Setlow (5) on S. cerevisiae tends to rule out the second hypothesis. They report that photoreactivability is proportional to the amount of photoreactivating enzyme per cell. However, since the enzyme concentration and photoreactivability was compared between log phase and stationary phase cultures this correlation may be the result of other differences in the cells. The decrease in photoreactivability observed for the log phase culture was considered to be associated with a lower specific activity of the photoreactivating enzyme. Alternately, the decrease may be attributed to the DNA synthesis which occurs in log phase but not in stationary cells. In Section III. D. 2. it was found that the onset of DNA synthesis was accompanied by a decrease in the photoreactivable fraction for UV-irradiated uvs cells.

V. RESULTS: INDUCTION OF FORWARD MUTATIONS AND THE EFFECTS OF REPAIR

A. Introduction

By analyzing the induction of forward mutations it is possible to evaluate the general mutagenic action of UV. After irradiation cells are plated to YEPD; thus, nearly all induced supplementable mutations can be recovered among the survivors. Since the mutants produced by all types of primary lesions are recoverable, the general mutagenic action of UV can be determined. It is, therefore, possible to assess whether UV-induced lesions in general and UV-induced dimers, specifically, act primarily to produce addition-deletion or base-pair sugstitutions mutations. Furthermore, mutations in the uvs strain can be compared to those in the UVS strain to determine if repair appreciably alters the spectrum of induced mutants.

The forward mutants to be studied in depth were those induced at the tryptophan synthetase gene, the tr 5 locus. The enzyme product of this gene catalyzes the addition of serine to indole-3-glycerol-phosphate to produce tryptophan (83). The tr 5 locus was chosen for study because of the extensive biochemical and genetic examination of other mutants of this gene (83).

B. Procedure

1. Characterization of Induced Mutants

To determine whether a mutation arose by either addition or deletion of bases or the substitution of a base, the tr 5 mutants were classified as follows: capable of complementing, osmotically remedial, or supersuppressible. The number of mutants exhibiting at least one of these properties was considered to be a minimum estimate

of the total mutants resulting from base-pair substitution (BPS) events. The reasons for such an assumption are presented in the following discussion.

Intragenic complementation occurs if a diploid heteroallelic for two mutants at a locus exhibits growth on the corresponding omission medium. Complementation occurs by the interaction of the altered monomeric protein products of these mutants to produce a functional oligomer complex (30, 111; for review see 29: pp. 62-89). Crick and Orgel (20) propose that the interaction which restores partial activity to the oligomer complex is thought to be "...the correction of the misfolding of one monomer (produced by the mutation) by some unaltered part of the other monomer".

The assumed mechanism of intragenic complementation strongly suggests that complementing mutants arise principally by base-pair substitution events of the missense type. The other classes of mutants, those due to either addition-deletion (AD) or BPS of the nonsense category, would be expected to complement only rarely. The polypeptide product of the gene that had an AD mutation in it would be completely in error for all amino acid codons translated beyond the corresponding AD site in the gene. A nonsense mutation in a gene would prevent any translation beyond the corresponding nonsense codon, the result being a polypeptide fragment (85). For complementation to occur there must be complementary regions of functional polypeptide in the two "mutant" monomers. Two AD mutants would have large non-complementary regions of faulty protein, and, therefore, the interaction leading to complementation could not take place. Similarly, two nonsense mutations would lack homologous regions of the protein and should not

complement. Thus, mutants that complement are generally considered to be the result of BPS events of the missense type. The exception would be an AD or nonsense mutant at the end of a gene which might conceivably complement a BPS mutant at a location in the gene that was translated earlier. This could occur if the information beyond the AD or nonsense mutant site was not critical in the oligomer interaction of the mutant polypeptides. However, only polarized complementation would be expected. BPS mutants in the region beyond the AD or nonsense site could not complement.

Osmotic remediability has been extensively examined by Hawthorne and Friis (48). Osmotic remedial mutants have their requirements alleviated by a change in osmolar condition of the media. These mutants have the properties of other known BPS mutants; many complement or are temperature sensitive, and are concluded to arise by base-pair substitution events.

Super-suppressibility of the mutant sites and the BPS origin of such alleles is presented in Section VI. B. To summarize, a suppressible allele is one that contains a codon (nonsense codon) which terminates translation. The suppressor product is assumed to be an altered tRNA which recognizes this nonsense codon in the messenger RNA and permits translation to continue. If the nonsense codon resulted from an AD mutation, translation past the nonsense site would yield a faulty protein. The reading of the codons would be shifted by the AD event and thus the translation would be incorrect.

Although these are useful methods for classifying the effect of the primary mutational lesion on the DNA, the observed numbers of BPS mutants are only minimum estimates. Another group of predominantly

BPS mutants, those which are leaky, were excluded in these experiments because of the difficulty in distinguishing them from the wild type. Since leaky mutants have corresponding proteins that still retain some function, in most cases they are probably not due to the addition or deletion of bases or to the presence of a nonsense mutation.

It is possible, however, that a leaky mutant could arise by the net addition-deletion of an integral number of codons (0, 1, 2, etc.), thus, the order of reading would be maintained. Such a leaky mutant has been identified in E. coli. The protein of a revertant at the tryptophan synthetase gene exhibited lower activity than the wild type (7). In a sense, therefore, the revertant was still a mutant; however, it was leaky to the point of almost appearing as wild type. The revertant was the result of a base addition at one position and a base deletion at another. An AD or nonsense mutant might also be leaky if the mutation were close to the translation terminating end of a gene, providing that this region was not critical to the protein's function. Such is the case with the tr 5-29 allele in yeast reported by Manney (83, 84). This mutant is suppressible, leaky (personal communication), and osmotically remedial. Furthermore, it is located near the gene end that corresponds to the termination of translation.

In the present study AD or nonsense mutants are considered to represent a small fraction of the leaky mutants. Further evidence for this assumption is the high incidence of leaky mutants which exhibit non-polarized complementation (146). Since the leaky class is considered to arise almost entirely from BPS mutational events, the numbers of BPS mutations observed in the non-leaky class - the one under study - represent only the lower limit to the possible BPS

mutants inducible by UV.

2. Isolation of Mutants

All experiments were conducted in a room maintained at 30°C. The strains used were KC614-6-16C (UVS) and KC614-6-24B (uvs) (Section VI. C.). Prior to irradiation, cells were incubated in YEPD for four to six days. The cells were resuspended in buffer and plated as a single cell layer on buffered agar discs (Section II. B.). After the liquid was absorbed by the agar the cells were irradiated with UV. Photoreactivation was then carried out with a high intensity blue light source (Section II. D.). After treatment the cells on the agar discs were resuspended in buffer, diluted, and plated to YEPD + TR. The dilution was adjusted to yield approximately 100 to 300 colonies per plate. These plates were incubated for three days.

The colonies that appeared after three days were replica-plated to synthetic complete (C) and to C - TR + Anthranillac acid (AN, 20 mg/liter). Only mutants that exhibited no growth at 2 days on either of the synthetic media were examined further. At this time only non-leaky mutants were detectable. Some of the mutants grew on C but not on C - TR + AN and were, therefore, presumptive tryptophan (tr) mutants. Others failed to grow on C and apparently had a requirement for some nutrient not present in this medium. These mutants were termed "unclassified".

Mutants capable of growing on C but not on C - TR + AN should include only mutations at one of the following loci: tr 4, tr 1, tr 3, or tr 5. The enzyme products of these loci control respectively the four steps in biosynthesis of tryptophan from anthranillac acid (22, 24). These mutants were tested further to determine the locus involved by

crossing them with known non-complementing tr 4, tr 1, tr 3, and tr 5 mutants.

Allelism tests were performed by mass mating. Sixteen mutants were streaked on a YEPD plate. A lawn of a tester was grown on an individual YEPD plate. After two days growth the mutant streaks were replica-plated to a new YEPD plate, and the tester was then replica-plated to this same plate. Mating and growth were allowed for one day, after which this plate was replica-plated to C - TR media. Inability of a mutant X tester inoculum to grow on C - TR indicated that the mutant was at the tester locus.

Intragenic complementation tests were performed in a similar manner on the mutants determined to be at the tr 5 locus. The testers in this case were tr 5 mutants that represented known complementing classes at this locus. The lack of growth of a mutant X tester inoculum on the C - TR medium was considered as evidence for location of the mutant in the complon being tested. A complementation map based on that presented by Manney (83) for these tr 5 complementing mutants is shown in Fig. 11; tr 5-47 has been added to this map.

To test for suppressibility the tr 5 mutants were streaked on to YEPD, incubated for two days, and replica-plated to three C-HI-LY plates. One plate was irradiated with UV and another with X-ray so as to induce super-suppressors (36). The other plate received no irradiation. Since hi 5-2 and ly 1-1 are super-suppressible, the spontaneous or induced revertant colonies appearing after five days were considered to be due to super-suppressors rather than independent reversions at the two loci. These colonies were then replica-plated to C - TR. If any revertant on the C-HI-LY plate also grew on the C - TR,

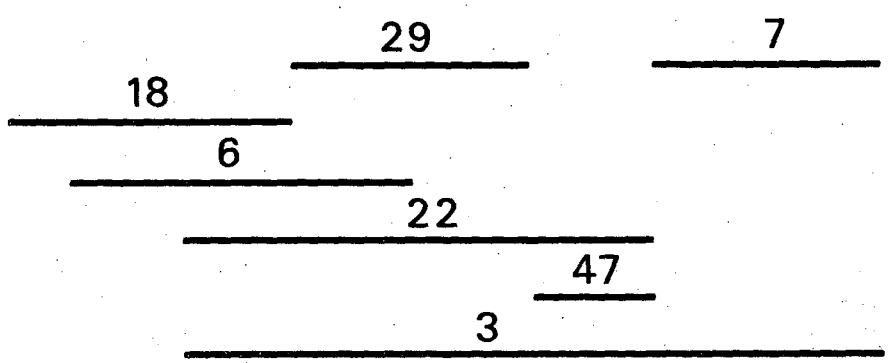

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Figure 11. Complementation map of tr 5 mutants (Manney (83) and Mortimer, personal communication). Overlapping lines indicate that the corresponding mutants do not complement.

then the corresponding tr mutant was considered super-suppressible. If none of the colonies on the C-HI-LY plate would grow on C - TR, the mutant might still be suppressible, since not all possible super-suppressors are selected by this technique (37). The results are only a minimum estimate of the number of mutants which were super-suppressible.

Mutants considered to be osmotically remedial could grow on media of altered osmolarity. That is, no growth appeared on C - TR media. However, growth of the osmotic remedial mutants was sustained if they were transferred to C - TR plates containing 0.5, 1.0, or 1.5 M NaCl.

In these experiments mutants unable to grow on synthetic complete medium also were examined. These mutants might be satisfied by any of a number of nutrients that are present in YEPD but not in the synthetic complete medium. However, from experience with similar mutants in other studies (Mortimer, personal communications) these mutants likely involve one of the following amino acid biosynthetic pathways: isoleucine-valine, tyrosine-phenylalanine, glutamic acid, or proline. The mutants unable to grow on the synthetic complete medium are an additional sample of UV-induced mutants at loci distributed throughout the genome (88). When possible, mutation induction at the tr 5 locus was compared with induction of unclassified mutants to determine whether UV mutagenesis at tr 5 was representative of mutagenesis in other parts of the genome.

C. Results

1. Forward Mutation Induction - uvs Strain

To determine whether pyrimidine dimers contribute appreciably to forward mutation, experiments were conducted at one UV dose (27 ergs/ mm^2) with and without PR. The results of this study with regard to

TABLE 10

Forward mutation induction in KC614-6-24B (uvs 9-3)

UV dose: 27 ergs/mm^2

			Tryptophan mutants							Unclassified mutants	
					Mutant locus						
Exp.	PR or no PR	% survival	Total* clones	Total tr mutants	tr 5	tr 4	tr 3	tr 1	Unresolved	Total* clones	Total mutants
17	NP	24.0	12,950	3 (0.23)†	2 (0.15)	1 (0.08)	0	0	0	--	--
	PR	77.2	2,166	0	0	0	0	0	0	--	--
17b	NP	16.1	9,400	6 (0.64)	3 (0.32)	1 (0.11)	0	1 (0.11)	1 (0.11)	--	--
	PR	62.3	3,883	0	0	0	0	0	0	--	--
24	NP	8.7	7,163	8 (1.12)	4 (0.56)	2 (0.28)	0	1 (0.14)	1 (0.14)	--	--
	PR	32.2	8,607	0	0	0	0	0	0	--	--
32	NP	6.1	12,720	17 (1.34)	7 (0.55)	4 (0.31)	4 (0.31)	0	2 (0.16)	12,720	38 (2.99)
	PR	43.9	8,885	2 (0.23)	0	1 (0.11)	0	0	1 (0.11)	8,885	10 (1.13)
Total	NP	--	42,233	34 (0.81)	16 (0.38)	8 (0.19)	4 (0.09)	2 (0.05)	4 (0.09)	12,720	38 (2.99)
	PR	--	23,541	2 (0.09)	0	1 (0.04)	0	0	1 (0.04)	8,885	10 (1.13)

*Based on multiplying the average of three plates by the total number of plates examined.

†Frequency of mutants per 10^3 survivors.

mutation frequency are displayed in Table 10. Based on the amount of mutational damage that was photoreactivable (16/42,233 before PR; 0/23,541 after PR), UV mutagenesis at the tr 5 locus was found to occur primarily through the production of pyrimidine dimers. This result enabled determination of the mutagenic effect of these dimers.

It can be deduced that at least 75% (12/16) of the tr 5 mutants exhibiting a complete block arose by a base-pair substitution alteration (Tables 11 and 12). This percentage is a compilation of the number of mutants that have at least one of the following properties: osmotically remedial, suppressible, or capable of complementing. As noted in the introduction to this section, the percentage represents only the lower estimate of the amount of BPS damage. Since the mutations appear to be due mainly to pyrimidine dimers, the primary mutagenic effect of these dimers when present at the tr 5 locus can be considered as being the production of BPS mutations.

Based on these observations about the mutagenic effect of pyrimidine dimers it is possible to propose a scheme of how DNA replication is affected by the presence of these lesions. Although the dimer causes a kink in the DNA, the polymerase is not prevented from replicating past it. Presumably, if DNA synthesis were stopped, the cell would not reproduce, and the mutation could not be revealed. Thus the lesion would be lethal rather than mutational. Assuming then that replication past the pyrimidine dimer does occur, the polymerase probably replicates each base of the dimer rather than adding or omitting a base or bases. However, the interpretation of the bases in the dimer by the DNA polymerase might be incorrect, the result being a BPS mutation.

The lesions involved in producing mutants at the other tryptophan

TABLE 11

Characteristics of UV-induced tr 5 Mutants Isolated in Strains

KC614-6-16C (UVS) and KC614-6-24B (uvs 9-3)

UV-sensitivity	PR or no PR	Mutant Strain	Osmotic Remedial	Suppress-ible	Complementation with other tr 5 alleles: 5-3	5-6	5-7	5-18	5-22	5-29	5-47
uvs	NP	T1	+	+	-	+	+	-	+	-	-
		T5	-	-	-	-	+	-	-	-	-
		T6	-	+	-	-	+	-	-	-	-
		T7	-	+	+	+	+	+	+	+	-
		T8	-	-	-	-	-	-	-	-	-
		T11	-	-	-	-	+	-	-	-	-
		T16	-	+	-	-	-	-	-	-	-
		T17	+	+	-	+	+	+	+	+	-
		T19	-	-	-	-	-	-	-	-	-
		T20	-	-	-	-	+	-	-	-	+
		T22	-	+	-	-	-	-	-	-	-
		T27	-	-	-	-	+	-	-	-	+
		T29	+	-	-	-	+	-	-	-	+
		T30	-	-	-	-	-	-	-	-	-

TABLE 11 (continued)

UV-sensitivity	PR or no PR	Mutant Strain	Osmotic Remedial	Suppress-ible	Complementation with other tr 5 alleles: 5-3	5-6	5-7	5-18	5-22	5-29	5-47
uvs	NP	T31	-	-	-	-	-	-	-	-	-
		T33	-	+	-	-	-	-	-	-	-
	PR	none									
UVS	NP	T300	-	-	-	-	+	-	-	-	-
		T301	-	-	-	-	+	-	-	-	-
		T303	-	-	-	-	+	-	-	-	-
		T305	-	+	-	-	-	-	-	-	-
		T306	-	-	-	-	-	-	-	-	-
		T307	-	-	-	-	+	-	-	-	-
		T308	-	-	-	-	-	-	-	-	-
		T310	-	-	-	+	-	+	+	+	+
		T311	-	-	+	+	+	-	+	-	+
		T315	-	+	-	-	-	-	-	-	-
		T316	-	-	-	+	+	+	-	-	-
		T317	-	-	-	+	+	+	-	-	-
		T319	+	-	-	+	+	+	+	-	+

TABLE 11 (continued)

UV-sensitivity	PR or no PR	Mutant Strain	Osmotic Remedial	Suppress-ible	Complementation with other tr 5 alleles: 5-3	5-6	5-7	5-18	5-22	5-29	5-47
UVS	PR	T400	-	+	-	-	+	-	-	-	-
		T403	+	-	-	+	+	-	+	+	+
		T406	+	+	-	+	+	-	-	+	-
		T408	-	-	-	-	-	-	-	-	-
		T409	-	-	+	+	+	-	+	-	+
		T413	-	+	-	-	-	-	-	-	-

Table 12

Summary of UV-induced tr 5 mutants isolated in strains KC614-6-16C (UVS) and KC614-6-24B (uvs 9-3)

Mutant Classification				uvs 9-3		UVS	
Category	Suppressible	Complementing	Osmotic Remedial	NP	PR	NP	PR
1	+	+	+	2	0	0	1
2	+	+	-	2	0	0	0
3	+	-	-	3	0	2	1
4	+	-	+	0	0	0	0
5	-	+	+	1	0	1	1
6	-	-	+	0	0	0	0
7	-	+	-	4	0	8	2
8	-	-	-	4	0	2	1
Minimum percent of tr 5 mutants due to base-pair substitution mutations (categories 1-7):				75	--	85	83

loci were also mainly (90%) pyrimidine dimers. This was consistent with the tr 5 results. Although a large amount of the damage that leads to "unclassified" mutants is photoreactivable (62%) the proportion is less than for tryptophan mutant induction. This could be a reflection of differences in the DNA composition of the tryptophan genes as compared to other parts of the genome. Possibly there is less chance for pyrimidine dimers to form due to fewer adjacent pyrimidines. Of course, it is also possible that dimers occuring at the tr loci are more accessible to the photoreactivating enzyme than those in the "unclassified" loci.

Although UV is able to produce non-photoreactivable damage in other parts of the genome, at the tr 5 locus its mutagenic effect in the uvs strain is mainly the induction of pyrimidine dimers. Dimers at this locus primarily cause base-pair substitution alterations.

2. Forward Mutation Induction - UVS Strain

Results in these experiments were different than for the uvs strain, although the survival levels were comparable. Quantitatively the amount of damage for the tr loci was much less, about one-third the frequency observed for the sensitive strain (Table 13). Qualitatively the damage was also much different for these loci in that no photoreactivation of mutations was detected. Since the damage at all the tr loci and the tr 5 locus specifically was shown to be readily photoreactivable in the uvs strain, the tr mutants in the UVS strain can most likely be ascribed to arising from lesions other than pyrimidine dimers. Therefore, at equal levels of survival the damage remaining after repair in the UVS strain was different from that in the "repairless" strain. If pyrimidine dimers were a major

TABLE 13

Forward mutation induction in KC614-6-16C (UVS)

UV dose: 660 ergs/mm^2

			Tryptophan mutants							Unclassified mutants	
					Mutant locus						
Exp.	PR or no PR	% survival	Total* clones	Total tr mutants	tr 5	tr 4	tr 3	tr 1	Unresolved	Total* clones	Total mutants
19**	NP	10.0	6,040	3 (0.50)†	2 (0.33)	0	0	0	1 (0.17)	--	--
	PR	49.2	6,040	3 (0.50)	1 (0.17)	2 (0.33)	0	0	0	--	--
37	NP	15.6	15,561	8 (0.51)	6 (0.39)	1 (0.06)	0	1 (0.06)	0	15,561	48 (3.08)
44	NP	21.6	48,257	9 (0.19)	5 (0.10)	2 (0.04)	1 (0.02)	0	1 (0.02)	25,978	62 (2.38)
	PR	48.6	45,617	14 (0.31)	5 (0.11)	5 (0.11)	1 (0.02)	0	3 (0.07)	46,561	56 (1.23)
Total	NP	--	69,858	20 (0.29)	13 (0.19)	3 (0.04)	1 (0.01)	1 (0.01)	2 (0.03)	41,539	110 (2.64)
Total	PR	--	51,657	17 (0.33)	6 (0.12)	7 (0.14)	1 (0.02)	0	3 (0.06)	46,561	56 (1.23)

*Based on multiplying the average of three plates by the total number of plates examined.

**Although the dose in this preliminary experiment (780 ergs/mm^2) was different than in exp. 37 and 44, the results are comparable and are included.

†Frequency of mutants per 10^3 survivors.

component of damage at the higher doses applied to the UVS strain, they must have been removed very efficiently by the dark repair system. The lesions remaining that did cause mutations might have been unrepairable because of saturation of the repair system by other damage in the genome or because the lesions were not recognizable by the dark repair enzymes.

Although the mutational lesions in the resistant strain were primarily different than pyrimidine dimers, they gave rise to nearly the same frequency of base-pair substitution mutations observed in the uvs strain, 84% (16/19*) at the tr 5 locus (Table 11 and 12). Thus the non-photoreactivable damage in the UVS strain also acted primarily to cause BPS alterations at the particular survival level tested. Similarly the frequencies of the different classes of mutants (i.e. osmotic remedial, complementing and osmotic remedial) appeared comparable in many aspects to those in the uvs strain (Table 12).

Little overlap was observed in the complementation patterns of mutants obtained in the uvs and UVS strains. The tr 5 mutants that exhibited the coincident complementation patterns between the two strains were those that complemented only with tr 5-7, a mutant site close to the end of the gene (83). There was no homology of complementation response for the other seven (out of ten) mutants in the sensitive strain with the remaining nine (out of thirteen) in the UVS strain. This may have been the result of the susceptibility of different regions to mutation by the primary lesions, which were not

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*Because the damage in the UVS strain was presumed to be other than pyrimidine dimers the results with and without PR were added together.

alike, in the two strains at equal survival levels. Or repair might be more active in certain areas of the tr 5 gene. When complementation in the uvs strain was compared to the results obtained by Manney in another UVS strain (83), a large overlap in the pattern of complementation occurred. Such a comparison of data between results in the present experiment and those obtained by Manney is done with reservation since the experimental conditions, as well as the strains, were quite different. Hence the internal comparisons of the present experiments are probably more valid as the strains were nearly isogenic and the irradiation conditions were identical except for UV dose.

Within the complementation groups of the two strains a trend of difference in suppressor mutation induction appears. Four of the ten complementers in the uvs strain were suppressible, whereas only one in thirteen were suppressible in the UVS strain. (The p-value of the chi-square test for homogeneity is $.05 < p < .10$). It is of interest that a similar result (1/19) was obtained by Manney (84) in another UVS strain. (If the results of this and Manney's study are added together the difference is quite large (4/10 Vs. 2/32).) As noted in Section V. B., complementing mutants that are suppressible most likely occur near the end of the gene. Thus the difference in the relative induction of these mutants in the uvs and UVS strains may prove important for understanding preferential repair at the genic level. Alternately it may be a reflection of the susceptibility of different parts of the gene to the various lesions induced in the uvs and the UVS strains, as suggested above for differences in complementation patterns.

3. Sectoring of Forward Mutants in the uvs and UVS Strains

UV mutagenesis in the two strains differed in yet another way

from those already mentioned: sectoring of mutational damage was found to be much higher in the sensitive strain. Sectoring is evidenced by the presence of both wild-type and mutant cells in a clone. The two types of cells are not mixed but appear as discrete sectors, presumably a result of segregation of mutational damage during the first few cell divisions after irradiation. The following diagrams describe the appearance* of some sectored colonies after replica-plating to the appropriate omission medium (the blackened areas indicate no growth):

whole mutant 1/2 sector 1/4 sector 1/8 sector

At least 50% of the forward mutants induced in the sensitive strain, both tr 5 and unclassified, were isolated from such sectored colonies (Table 14). Contrary to this, most of the mutants (greater than 80%) of the wild type strain came from "whole mutant" colonies.

To understand the difference in the frequency of UV-induced whole colony mutants between the two strains, it is helpful to examine how the mutants may arise. A priori whole colony mutants are not expected if a lesion is induced in only one rather than both strands of DNA. After DNA synthesis and cell division one cell would be expected to have "mutant" DNA and the other normal. Thus, the resulting colony would be sectored. To explain the frequent occurrence of whole

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*For colonies that are mutant, these patterns are easily discernible after a few days growth, if care is taken when replica-plating to omission media. During replica-plating the two plates should be firmly pressed against the velveteen cloth and then removed with a flick of the wrist.

Table 14

Sectoring of UV-induced tr 5 and Unclassified Mutants

Mutant Class	Expt.	Strain	PR or No PR	Whole Colony Mutants	Sectored Colony Mutants	% Sectored Colonies	Size of Mutant Sector		
							1/4 or less	1/4 to 1/2	3/4
uncl.	32	uvs	NP	20	18	47	5	13	0
"			PR	3	7	70	5	1	1
"	37	UVS	NP	40	7	15	2	2	3
"	44		NP	51	10	16	1	8	1
			PR	44	12	21	2	8	2
tr 5	--	uvs	No PR	8	8	50	1	7	0
"	--	UVS	No PR	12	1	21*	1	0	0
"	--		PR	3	3		0	3	0

*Results with and without PR are combined (see text).

colony mutants, Haefner (44, 45) proposes that pure mutant clones probably originate either (1) by UV inducing damage that affects both strands of the DNA and (2) by repair acting on the unaffected strand such that when new DNA was being synthesized opposite the area of the lesion, incorrect bases that more appropriately pair with the lesion bases would be added. These hypotheses are based on the results of pedigree analysis of UV-irradiated cells of Schizosaccharomyces pombe; among these cells were UV-induced mutants that would have given rise to either sectored or whole colony mutants if they were not pedigreed. He excludes as an explanation for whole colony mutants the possibility of a master strand for replication on the basis that many mutant colonies were sectored. The possibility of lethal sectoring was found to be unlikely since some pedigrees in which all the cells were mutant exhibited little or no lethal sectoring. Also improbable is the induction of independent lesions on opposite strands of the same gene; Haefner could detect no intragenic recombination between branches of the same pedigree.

The results of the present study are inconsistent with the expectations of the first hypothesis proposed by Haefner (damage induced in both strands). From the amount of mutational damage that was photoreactivable, it can be concluded that one-half of the UV-induced unclassified mutants arose by the production of pyrimidine dimers. Since 80% of the mutants induced in the absence of PR were isolated as whole colonies and the relative frequency of whole colony mutants before and after PR was the same, a minimum of 40% ($.5 \times .8$) of these whole colony mutants were induced by these pyrimidine dimers. As shown by Wacker (137) pyrimidine dimers occur between adjacent bases

in the same single strand of DNA at biologically meaningful doses. Thus, for at least 40% of the mutants the proposition is ruled out that whole colony mutants are a consequence of damage that affects both strands of the DNA.

The other hypothesis suggested by Haefner is based on the action of repair to produce whole colony mutants. If repair converts potentially sectored-colony mutants to whole colonies, then most of the mutant colonies originating from a UV-irradiated repairless (uvs) strain should be sectored. As mentioned earlier at least 50% of the mutant colonies from such strains were sectored. It is conceivable that the reason that a larger percentage of sectored colonies was not observed was related to lethal sectoring in yeast (43).

As noted above Haefner rules out lethal sectoring as a possible reason for lack of mutant sectoring in the wild-type strains of Schizosaccharomyces pombe (45). However, UV-sensitive strains of this yeast (45) and of E. coli (46) have been shown to exhibit much larger frequencies (2 and 3 times) of lethal sectoring after UV when compared to the wild type. At the 14% survival level, 50% of the UV irradiated yeast cells (S. pombe) exhibited a first division lethal sector. That is, only one of the two cells after the first division following UV treatment formed a colony. A comparable level of survival would have resulted in about 40% (based on extrapolation of reported values) first division lethal sectoring in E. coli B_{s-1} (46). In UV-sensitive strains of S. cerevisiae there is also a "marked increase" (46) of lethal sectoring after irradiation when compared to the wild type. Although data were not presented, it is reasonable to assume that the frequency of first division lethal sectoring in S. cerevisiae is

comparable, 40-50%. As proposed earlier, the irradiated cells of a repairless strain should yield only sectored colonies. However, 40-50% of the cells would be expected to exhibit first generation lethal sectoring. Thus, about 50% of the mutants induced in a repairless strain of S. cerevisiae would be whole colony. About this frequency was observed for the tr 5 and the "unclassified" mutants (Table 14).

Therefore, the results are consistent with the hypothesis that the appearance of whole colony mutants in the UVS strain is a repair-dependent phenomenon. Even though the major function of repair is to remove lethal and mutational lesions, it can be proposed that it occasionally acts on the strand opposite to the lesion. In resynthesizing the excised region opposite the damage, bases may be added which more appropriately match the new hydrogen bonding properties of the bases in the lesion, thus yielding an altered strand of DNA opposite the dimer. If the dimer were then repaired in the same or subsequent cell generations (9), the template for repair would be the altered strand. Presumably if DNA synthesis occurred before repair of the dimer took place, the bases added opposite the dimer would be the same ones present in the altered strand. Consequently both strands would be changed and after several divisions a whole colony mutant would be observed.

VI. RESULTS: UV-INDUCED REVERSION OF MUTATIONS AND THE EFFECTS OF REPAIR

A. Introduction

The ability of UV radiation to produce mutations and the role of repair in this process can be determined by examining the effectiveness with which UV reverts known mutations. In these experiments UV-induced

reversion to prototrophy in a repairless and a repair-sufficient strain was examined. The mutagenic action of UV was assessed by determining how effectively it could reverse addition-deletion (AD) and different types of base-pair substitution (BPS) mutants. Also the role of UV-induced pyrimidine dimers in causing reversion was assessed by performing experiments with and without PR.

B. Characteristics of Mutants Employed in Reversion Studies

Included among the base-pair substitution mutants were those due to missense and nonsense mutations. These types of mutants originate by a change in a single codon. Detectable missense mutants are a consequence of a codon being altered so that it codes for a new amino acid at a critical site in the protein, such that the polypeptide product of the gene is no longer functional.

A nonsense mutation occurs as a result of a change in a DNA triplet to one of the following: ATC, ATT, or ACT. The presence of the corresponding codons UAG, UAA and UGA in the mRNA leads to chain termination (35, 85) since the nonsense codon does not code for an amino acid. It is presumed that if a lesion is to revert a nonsense triplet it must occur at or near the position of this triplet. Phenotypic reversion of a nonsense mutation can also occur by the induction of a super-suppressor. One group of super-suppressors consists of tRNA genes in which the triplet corresponding to the tRNA anticodon has been altered so that a nonsense codon can be recognized (39, 41). Translation of mRNA's can then proceed past a nonsense codon and a polypeptide will be made. The mutational lesion that changes a tRNA so that it recognizes a nonsense codon is presumed to occur at or

near the region that transcribes the tRNA anticodon. Thus by studying reversion of nonsense mutations and the induction of their suppressors, the effect of UV radiation on a localized region of DNA can be determined.

The location of the lesion that leads to reversion of addition-deletion or missense mutations need not be as specific as that of nonsense mutations. Reversion of the AD mutation can occur either at the original mutant site or at another position in the gene as long as the original reading frame is restored (7). Similarly, the missense mutation can be reverted by a mutation at the original site, or at another site in the gene provided the two mutant sites code for amino acids that will return functionality to the protein. Although the location of the reverting lesion is not as specific as for the reversion of nonsense mutations, the effectiveness of UV in producing AD or BPS reversions of known AD or BPS mutations can be studied.

Among the mutant alleles examined for reversion were three of the base-pair substitution type, one missense, two nonsense, and one addition-deletion mutation:

<u>ly 1-1</u>, <u>hi 5-2</u>

These are nonsense mutations and have been reported to be suppressible by several classes of ochre super-suppressors (37, 51). The presence of these two alleles in a strain provided a convenient system for determining whether reversion of the lysine or histidine requirement occurred at the locus or by the induction of a suppressor. To test the type of reversion, the treated cells were plated on to either C-LY or C-HI. Colonies that arose on these plates were replica-plated to the complementary plate (i.e., C-LY to C-HI). If growth

of a colony imprint occurred on both plates, then the origin of the reversion was considered to be due to the induction of a super-suppressor. Since loss of either the histidine or lysine requirement is primarily due to reversion at the corresponding locus (77) rather than the presence of a suppressor that suppresses only one of the requirements (37), those colonies that would grow only on C-HI or on C-LY were classified as revertants at the locus.

thr 4-1

This mutation was considered to have arisen by an addition-deletion event. The arguments to justify this assumption are based on observations considered to be associated with AD mutants.

The thr 4-1 allele has been reported to have a higher reversion rate during meiosis compared to mitosis (75). This phenomenon, known as the "meiotic effect" is associated with recombination and has been suggested to be due to an unequal crossing-over during meiosis (78). The enhanced reversion frequency of hi 1-1 produced by the presence of an acridine during meiosis (79) lends further support to this hypothesis. Since acridines cause addition-deletion mutations, and hi 1-1 exhibits the "meiotic effect", mutants that show a "meiotic effect" are probably addition-deletion mutants. The effect of acridines on thr 4-1 under similar conditions has not been tested. Based on recombination tests designed to distinguish between reversion at the locus or by an external suppressor, Magni (75, 76) has concluded that reversion occurs only at the locus.

[le 1-12, ad 2-1

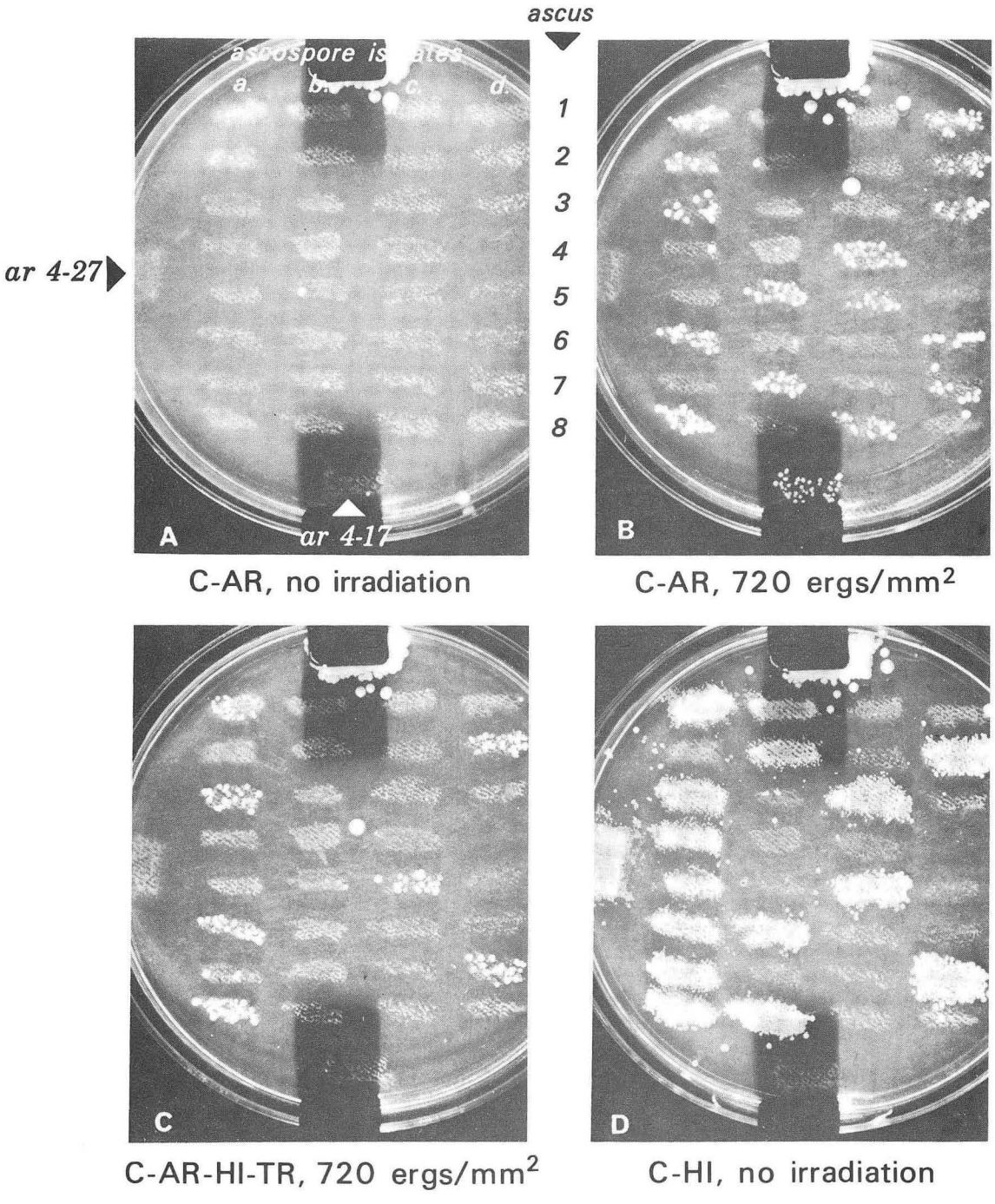
Reversions of the leucine and the adenine requirements were not examined in these studies. Based on the change in the le 1-12

reversion frequency (when present in a UVS strain) an additional alteration of the le 1 gene was considered to have occurred when uvs 9-3 was induced in X1687-101B. Another genic mutation for ad precluded further study of reversion of ad 2-1.]

ar 4-27

This mutant is considered to be a missense mutation since in crosses with other mutants of ar 4 it exhibits a non-polarized complementation pattern (Mortimer, personal communication), and ar 4-27 reverts at a high rate in the presence of hydroxylamine (77). Sora and Branduzzi (in preparation) observed that hydroxylamine, which is presumed to produce GC→AT transitions (11, 33, 81, 112), preferentially reverts missense mutants in yeast.

To generate a uvs haploid stock with ar 4-27, a strain marked by ar 4-27 was crossed with a uvs 9-3 ar 4-17 strain and a uvs 9-3 ar 4-27 spore isolate was selected. During the process of replacing ar 4-17 in the original strain by ar 4-27 a technique for distinguishing between the two alleles in spore isolates was developed. At low doses of UV (corresponding to about 10-20% survival) ar 4-17 reverts at a much higher rate than ar 4-27. Replica plates to different omission media of UVS parents (ar 4-17) and (ar 4-27) and the spore isolates of asci from this cross are included in Plate 1. In Plate 1A, few spontaneous arginine revertants are seen. On the C-AR plate that was UV-irradiated with 720 ergs/mm^2 (Plate 1B) a large number of revertants were induced on the ar 4-17 but not on the ar 4-27 parent streak. The high arginine revertibility inducible by UV segregated in a 2:2 ratio and was presumed to be due to induced



XBB 687-4327

Plate 1. The induction by UV of revertants in strains marked by ar 4-17 and ar 4-27 and in ascospore isolates from a cross involving these strains.

reversion of the ar 4-17 segregant. Since ar 4-17 is suppressible while ar 4-27 is not, the possibility of reversion occurring by the induction of super-suppressors was examined. As shown in Plate 1C (C-AR-HI-TR) there was no UV-induction of super-suppressors on the ar 4-17 parent streak. A large number of revertants appeared only on histidine independent spore streaks (Plate 1D). Other suppressible alleles tested (hi 5-2, ly 1-1) gave similar reversion rates. The suppressible mutant ar 4-4 has a UV-induced reversion rate about one-tenth that of ar 4-17 (31). This might be due to codon alterations at one site in the ar 4 gene being more effective than alterations at another. If the nonsense triplet for ar 4-17 and ar 4-4 is UAA (51) then reversion by either transition or transversion would lead to a codon that codes for one of five amino acids (lysine, glutamine, glutamic acid, tyrosine or serine) at the former nonsense site (18). If the reversion rates for transitions and transversion are similar and one allelic site is at a position such that only one amino acid will restore activity while any substitution can occur at the other, then an order of magnitude difference in revertibility might be expected between ar 4-4 and ar 4-17.

This method of determining the presence of a given allele is dose dependent. At a dose of 250 ergs/mm^2, or less, no allele discrimination was possible. Also, it was observed that the reversion rate in a homozygous diploid is about twice that for a haploid.

C. Procedure

To minimize the effects that genetic background might have on induced mutation rates (42) UV-sensitive and nonsensitive isolates of nearly the same genotype were used. The spore isolates KC614-6-16C

(α UVS ar 4-27 ly 1-1 le 1 hi 5-2 ad thr 4-1) and KC614-6-24B (α uvs 9-3 ar 4-27 ly 1-1 le 1 hi 5-2 ad thr 4-1) from the diploid KC614-6 were employed in this study. [In early experiments, 6 and 7, KC612-1 (α uvs 9-3 ar 4-17 ly 1-1 le 1 hi 5-2 ad thr 4-1) was used. In the rest of this study KC614-6-16C (UVS) and KC614-6-24B (uvs 9-3) were employed.] Irradiation and plating procedures are discussed in Section II. E. The UV-doses employed in the reversion studies are in terms of incident exposures to the irradiated cell suspensions. Since the titer of the cell suspensions was high (about 2×10^8 cells/cc) the actual dose to individual cells was much less. The DMF for irradiation in suspension vs. irradiation on agar (as a single cell layer is 18.5.

To calculate the induced reversion frequency for a given requirement, it was necessary to subtract the spontaneous frequency from the total frequency of revertants obtained after irradiation. The spontaneous (control) frequencies were determined by subjecting an unirradiated sample of cells to the same procedure as the irradiated cells. The number of revertants that arose on the control plates was divided by the number of viable cells on these plates to give the control reversion frequencies. Although the control reversion frequency estimates only the frequency of revertants that have accumulated in the population, the median value of the controls for a series of comparable experiments would be expected to approximate the spontaneous reversion frequency (74, 130). Little difference was observed between the controls of the sensitive and the resistant strains (Tables 15 and 18); thus, the spontaneous reversion frequencies of the loci tested are probably similar for these two strains.

Since the total induced suppressor or locus reversion frequencies were, in some cases, in the same order as the control frequency, it was possible for the induced frequency to be less than for the controls. For these cases the induced frequency was considered as being equal to one.

To determine whether results obtained without PR were significantly different from those obtained with PR, the following statistical analysis was employed. Generally, mutation frequency was an exponential function of dose or time of liquid holding. Therefore, the results were tested to determine if the same exponential function could describe both sets of data (no PR and PR). Least squares regression lines of log revertants/10^8 survivors vs. dose (or days in buffer) were evaluated for the case of no PR and the case of PR. A test was then made to determine whether a least squares regression line for all the data (no PR and PR) fit the data better than the individual regression lines (110). In this way the hypothesis that PR had no effect was tested. If there were an effect, then the probability of obtaining observations for which a least squares regression line for all the data fits better than the individual regression lines should be low. A p-value of less than .10 indicated that the results before and after PR were significantly different. Calculations were performed using the Biomedical Computer Program BMD 03R (23).

Inability to detect small differences may have been due to the variance of the data or an insufficient number of observations. In such cases the data was subjected to further statistical analysis. The smallest difference which could have been detected (at the $p = .05$ level of significance) with probability .8 was determined (110),

assuming that the average of the two variances (no PR and PR) was a good estimate of the actual variance. The minimum difference was determined for the middle dose or for five days holding recovery.

D. UV-induced Reversion in a uvs 9-3 Strain

In Table 15 and Fig. 13a-f are presented the results of UV-induced reversion studies at various loci in the uvs 9-3 strain. Included in these figures are results obtained with the wild type strain when irradiated at the low doses normally applied to the sensitive strain (data for the UVS strain are presented in Table 18). The survival of the uvs and UVS strains in the reversion experiments are shown in Table 16. It is apparent from these figures (13a-f) that UV induces reversion in all loci studied in the uvs 9-3 strain. However, neither the reversion levels nor the damage that causes reversion are the same for all loci. Reversion of base-pair substitution mutations is seen to vary considerably among ar 4-27, ly 1-1 (locus) and hi 5-2 (locus). In the absence of PR, the reversion frequency is lowest for ar 4-27 (approximately 10 to 100 revertants/10^8 survivors) and highest for the hi 5-2 locus (> 1000/10^8 survivors). Since repair of premutational damage presumably does not occur in the sensitive strain in these experiments (see following section), the differences in reversion of the BPS mutations may be attributed to one or more of the following: a) variation in susceptibility to the formation of UV-induced lesions at the various loci studied (for example, due to differences in base composition); b) variation in the number of codons which will lead to reversion (of the total number of codons to which a given codon can be changed, only a few may lead to reversion while for the other all may lead to reversion), and c) phenotypic expression of a reversional

TABLE 15 UV-induced Reversion Frequencies of Various Genes in a UV-sensitive Strain[1] (uvs 9-3)

Gene	Exp.	Dose	PR or No PR	Days in Buffer	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$) Locus	Suppressor
thr 4-1	6	0	-	0	1	0.77			1.3	
	14		-		0	0.446			0.0	
	18		-		1	9.00			0.1	
	27		-		2	3.48			0.6	
	33		-		0	0.59			0.0	
	14		-	5	0	0.648			0.0	
	18		-		-	-			(1.2)[5]	
	27		-		2	0.805			2.5	
	33		-		-	-			(1.2)[5]	
	33		-	10	-	-			(1.2)[5]	
	6	400	NP	0	42	2.1			18.7	
	18				24	1.4			17.0	
	27				17	1.6			10.1	
	7		PR		85	7.2			10.5	
	18				19	5.3			3.5	
	27				21	2.9			3.0	
	18	650	NP		23	0.46			49.9	
	27				13	0.26			49.4	
	18		PR		42	3.7			11.2	
	27				64	3.4			18.2	

TABLE 15 (continued)

Gene	Exp.	Dose	PR or No PR	Days in Buffer	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$) Locus	Suppressor
	14	850	NP		15	0.044			340.9	
	18				16	0.19			84.1	
	27				14	0.055			254.0	
	33				17	0.049			346.9	
	14		PR		68	0.67			101.5	
	18				57	2.3			24.7	
	27				42	1.7			24.1	
	33				117	1.7			68.8	
	14		NP	5	16	0.60			27.7	
	18				10	0.24			41.6	
	33				17	0.21			81.0	
	14		PR		63	3.7			17.0	
	18				58	4.07			13.0	
	33				122	4.8			24.2	
	33		NP	10	16	0.72			21.0	
	33		PR		79	5.7			12.6	
ar 4-27	18	0	-	0	1	6.75			0.1	
	27		-		1	3.50			0.3	
	33		-		0	0.59			0.0	
	14		-		0	0.64			0.0	
	18		-		-	-			(0.1)[5]	
	27		-		2	8.05			0.2	
	33		-		-	-			(0.1)[5]	

TABLE 15 (continued)

Gene	Exp.	Dose	PR or No PR	Days in Buffer	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$) Locus	Suppressor
	33		-	10	-	-			(0.1)[5]	
	18	450	NP	0	9	1.4			6.3	
	27				8	1.6			4.7	
	18		PR		8	5.3			1.4	
	27				6	5.9			0.7	
	18	650	NP		5	0.46			10.7	
	27				2	0.26			7.4	
	18		PR		11	3.7			2.8	
	27				17	3.4			4.7	
	18	850	NP		9	0.19			47.2	
	27				0	0.055			-0.3	
	33				3	0.033			90.9	
	18		PR		39	2.3			16.8	
	27				12	1.7			6.8	
	33				17	1.7			10.0	
	14		NP	5	5	0.60			8.3	
	18				1	0.24			4.0	
	33				1	0.20			4.9	

TABLE 15 (continued)

Gene	Exp.	Dose	PR or No PR	Days in Buffer	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$) Locus	Suppressor
	14		PR		24	3.7			6.5	
	18				16	4.1			3.8	
	33				11	4.8			2.2	
	33		NP	10	1	0.72			1.3	
	33		PR		3	5.7			0.8	
ly 1-1	6	0	-	0	38	0.77	38	37	1.3	48.1
	14		-		10	0.446	10	9	2.2	20.2
	18		-		30	0.45	11	11	0.0	66.7
	27		-		44	0.348	44	(43)[6]	2.9	123.6
	33		-		19	0.59	19	(18)[6]	1.7	30.5
	14		-		49	0.648	19	18	4.0	71.6
	18		-		-	-	-	-	(2.6)[5]	(48.2)[5]
	27		-		21	0.805	21	(20)[6]	1.2	24.8
	33		-		-	-	-	-	(2.6)[5]	(48.2)[5]
	33		-	10	-	-	-	-	(2.6)[5]	(48.2)[5]
	6	400	NP	0	88	0.21	88	17	289.2	80.5
	18				47	0.14	47	12	250.0	19.0
	27				43	0.14	43	13	140.0	40.7
	7		PR		153	0.72	153	64	122.3	40.8
	18				100	0.53	100	29	134.0	-12.0
	27				103	0.59	103	63	65.0	-16.8

TABLE 15 (continued)

Gene	Exp.	Dose	PR or No PR	Days in Buffer	Total Revertants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$)	
									Locus	Suppressor
	18	650	NP		130	0.46	118	42	182.0	33.9
	27				141	0.26	141	103	143.3	272.6
	18		PR		126	0.37	58	15	252.5	21.4
	27				77	0.34	37	2	100.1	0.0
	14	850	NP		18	0.044	17	6	262.5	124.2
	18				53	0.19	44	14	190.2	22.1
	27				44	0.055	44	29	269.8	403.7
	33				43	0.033	43	16	816.5	454.3
	14		PR		27	0.034	25	8	537.8	233.9
	18				99	0.23	99	(56)[5]	187.1	176.6
	27				67	0.17	67	31	208.9	58.8
	33				102	0.11	102	14	798.3	96.8
	14		NP	5	116	0.60	116	47	111.0	6.7
	18				74	0.24	74	(26)[5]	196.1	61.4
	33				49	0.14	43	16	218.5	82.0
	14		PR	5	196	0.27	116	13	640.6	9.7
	18				144	0.41	144	31	273.0	27.4
	33				146	0.32	102	14	392.4	14.4
	33		NP	10	62	0.48	30	5	105.0	-27.7
	33		PR	10	112	0.57	112	21	157.0	-11.4

TABLE 15 (continued)

Gene	Exp.	Dose	PR or No PR	Days in Buffer	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$) Locus	Suppressor
hi 5-2	18	0	-	0	31	0.45	31	30	2.2	66.7
	27		-		70	0.348	70	69	2.9	198.3
	33		-		78	0.59	28	27	1.7	45.8
	18	0	-	5	-	-	-	-	(2.3)[7]	(103.6)[7]
	27		-		-	-	-	-	(2.3)[7]	(103.6)[7]
	33		-		-	-	-	-	(2.3)[7]	(103.6)[7]
	33		-	10	-	-	-	-	(2.3)[7]	(103.6)[7]
	18	400	NP	0	247	0.14	247	(33)[5]	1526.3	169.1
	27				205	0.18	205	27	986.0	-48.3
	18		PR		903	0.53	431	18	1630.4	4.5
	27				68	0.059	68	8	1014.1	-62.7
	18	650	NP		182	0.046	89	2	3865.4	22.3
	27				108	0.026	105	7	3874.0	78.7
	18		PR		1122	0.37	550	6	2998.0	-33.6
	27				68	0.034	65	2	1935.6	-136.7
	18	850	NP		87	0.019	87	(4)[5]	4366.2	143.9
	27				65	0.0055	65	5	10906.1	710.9
	33				42	0.0049	42	1	8365.6	158.3

TABLE 15 (continued)

Gene	Exp.	Dose	PR or No PR	Days in Buffer	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$)	
									Locus	Suppressor
	18		PR		1443	0.23	1443	(10)[5]	6228.2	-23.1
	27				56	0.017	29	0	3291.2	-198.3
	33				72	0.011	72	1	6452.8	45.2
	18		NP	5	132	0.024	61	2	5317.4	76.8
	33				57	0.014	57	1	3997.7	32.1
	18		PR		1104	0.41	542	16	2772.3	-185.5
	27				113	0.052	113	(4)[5]	2093.9	-26.6
	33				70	0.032	70	3	2091.5	-9.8
	33		NP	10	142	0.048	142	3	2893.6	-41.1
	33		PR		101	0.057	101	2	1734.6	-68.5

[1]Strain KC614-6-24B (α ar 4-27 le 1 ly 1-1 hi 5-2 thr 4-1 ad uvs 9-3) was used for all experiments except 6 and 7, in which KC612-1 (α ar 4-17 le 1 ly 1-1 hi 5-2 ad thr 4-1 uvs 9-3) was employed.

[2]Total revertant colonies on the corresponding omission media plates.

[3]Estimated from the number of colonies on YEPD plates and the appropriate dilution factor.

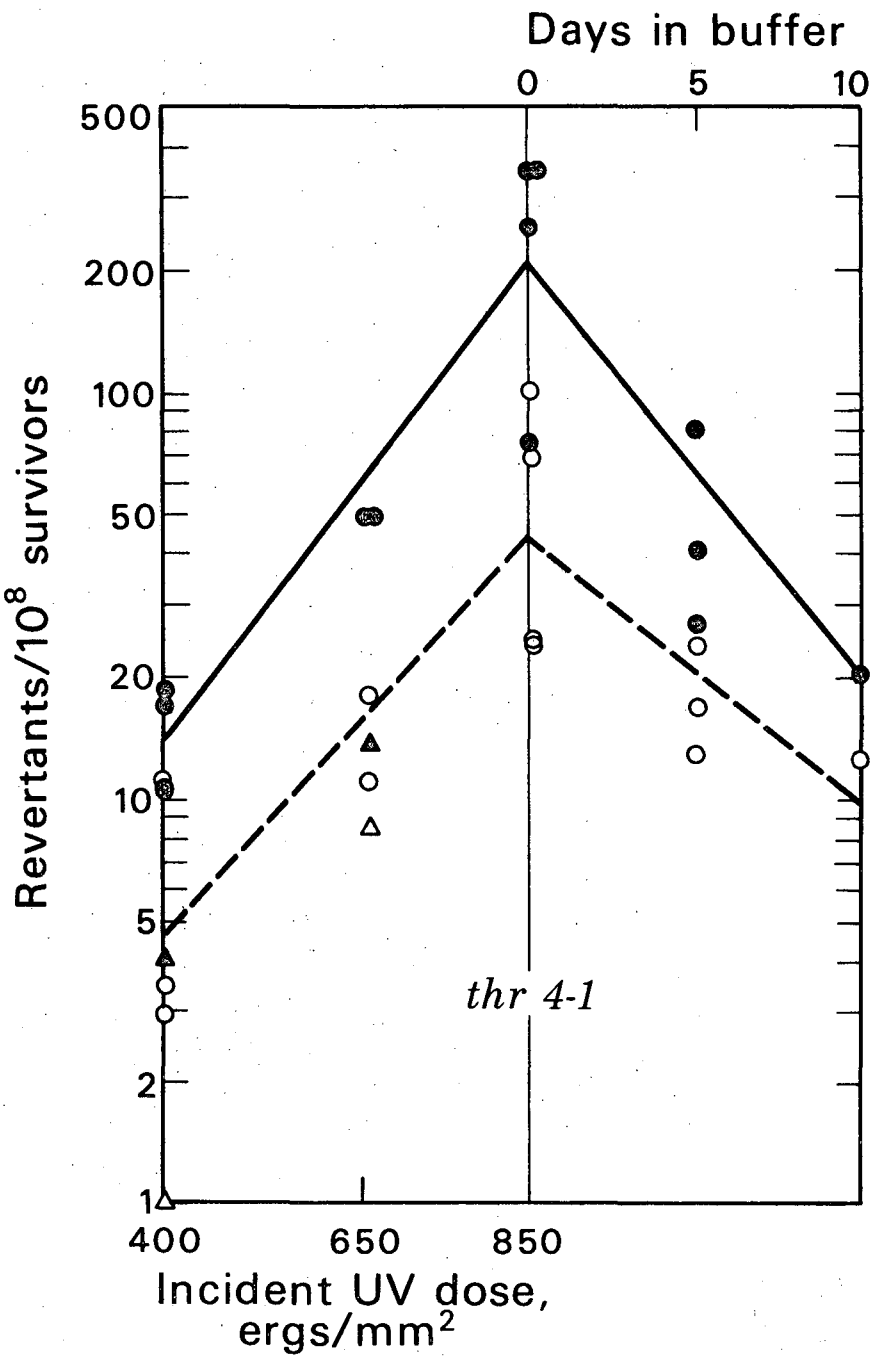
[4]Calculated induced reversion frequencies: Suppressor = (Total Revert./Viable Cells)[a] × (# Revert. due to Supp./# Reverts. Tested)[b] - Control. Locus = a × (1-b) - Control. An induced frequency that is zero or negative is assigned the value 1.0 in further calculations, e.g. for determining

the regression line of log x/y vs dose. The reversion of ar and thr is considered to occur at the locus only (see text).

[5]Sample was either contaminated or not measured; the value that appears is based on the average of values that correspond to measurements in other experiments performed under the given conditions. Day 10 control values are averages from Day 5 results.

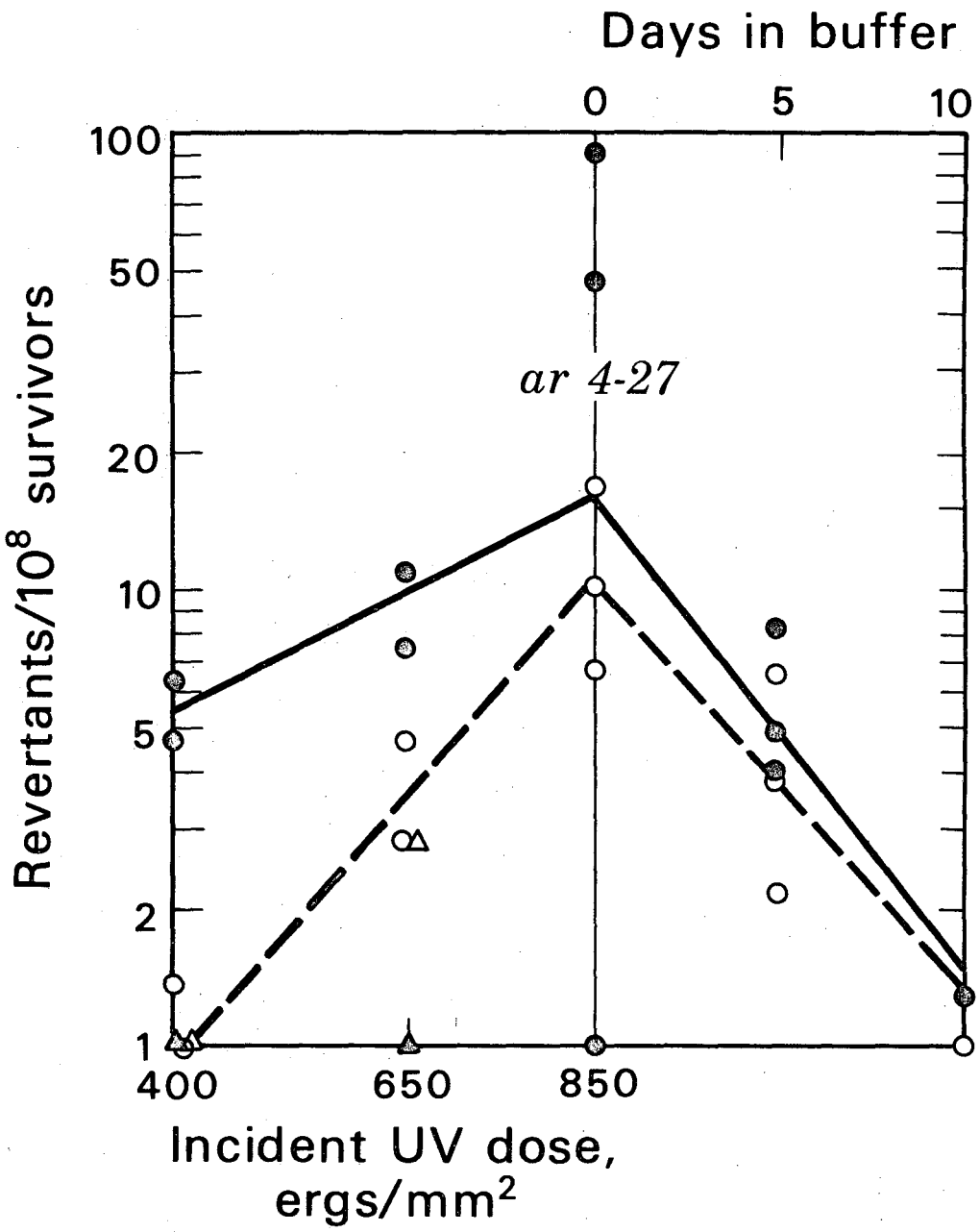
[6]Estimated value based on cases where the suppressor reversion frequency of ly 1-1 was measured (Exp. 6, 14, and 18).

[7]Average of Day 0 results.



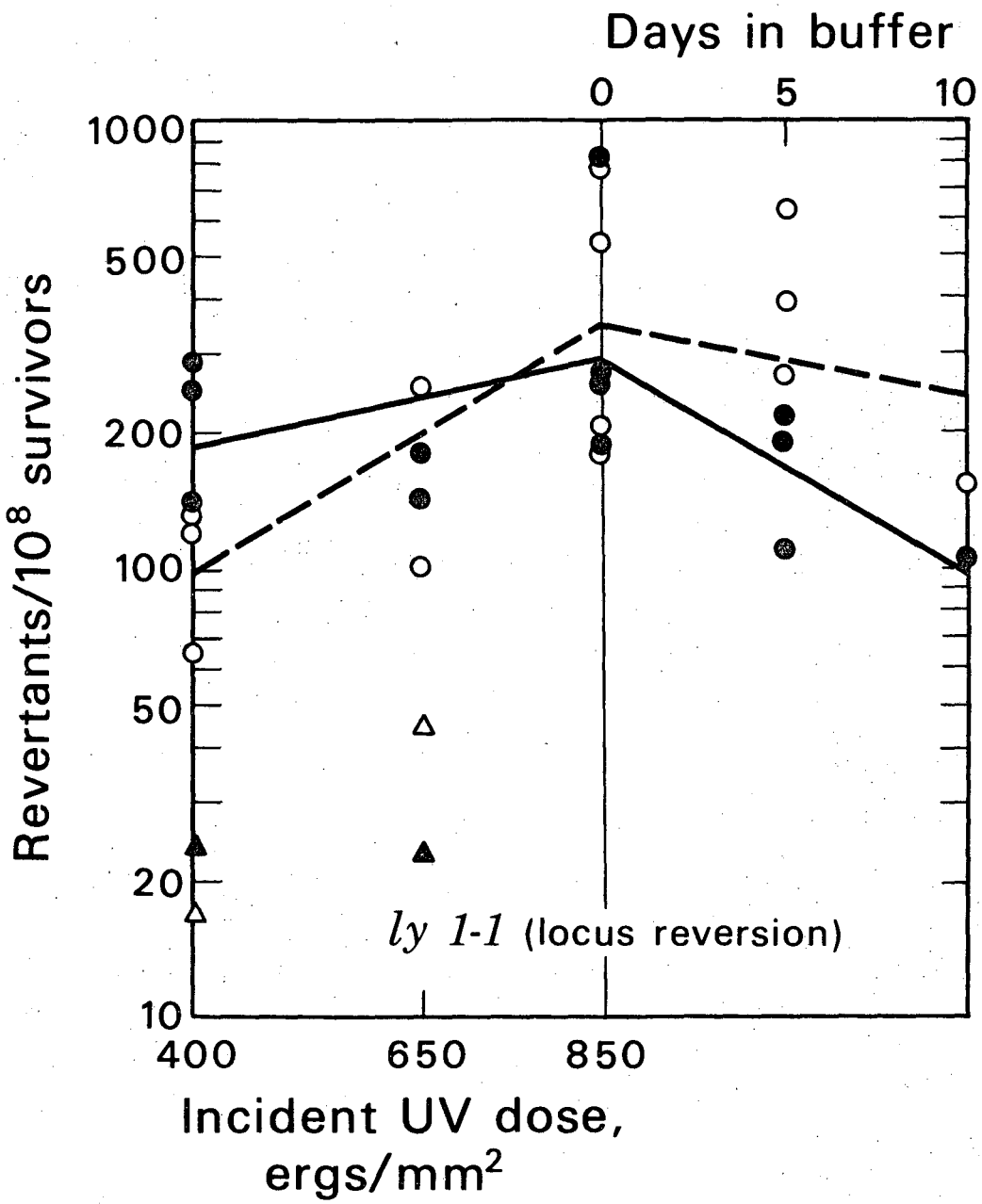
DBL 687-5345

Figure 13a. Reversion of thr 4-1 in a uvs 9-3 (circles) and a UVS (triangles) strain with (open symbols) and without (closed symbols) PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



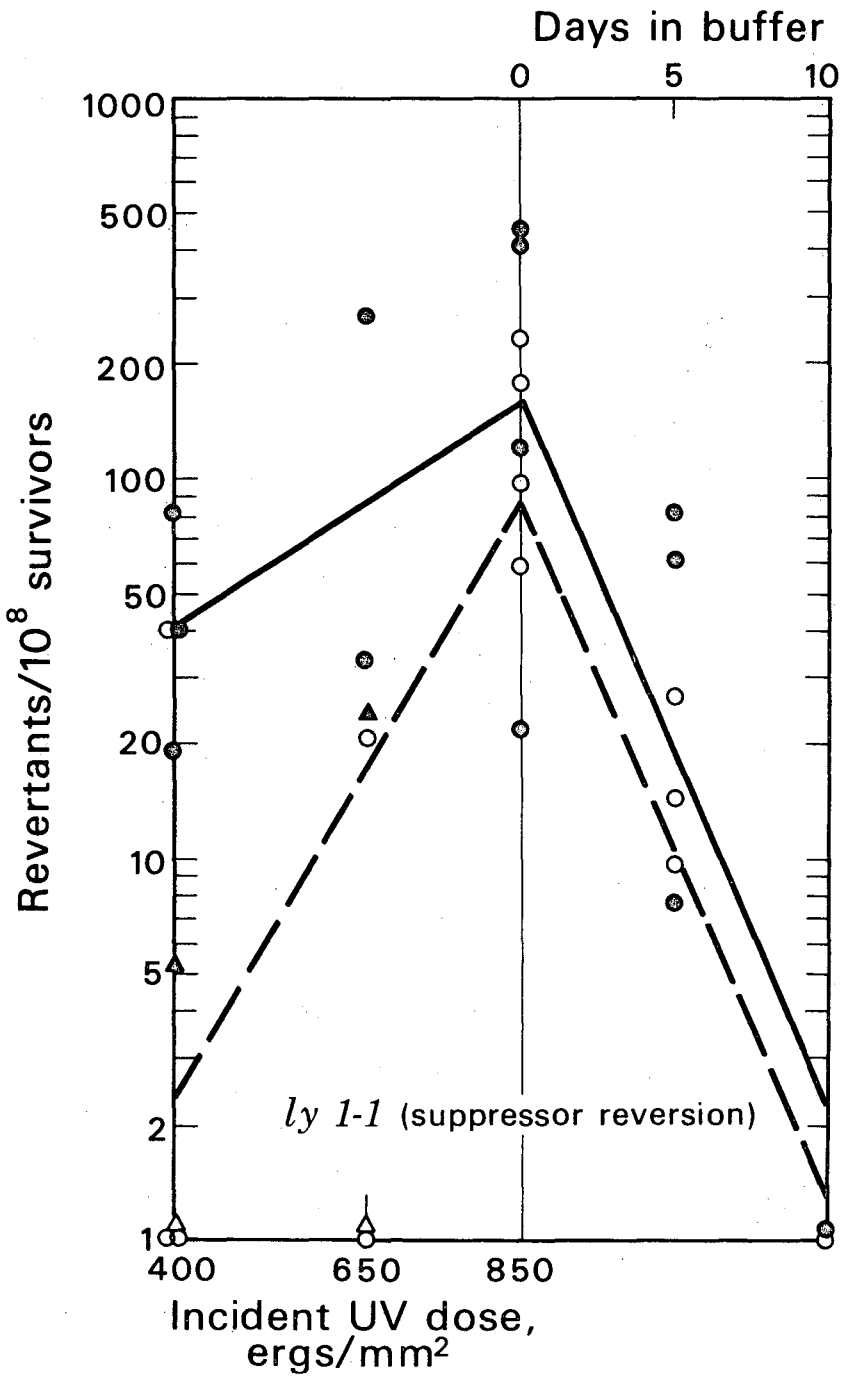
DBL 687-5343

Figure 13b. Reversion of ar 4-27 in a uvs 9-3 (circles) and a UVS (triangles) strain with (open symbols) and without (closed symbols) PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



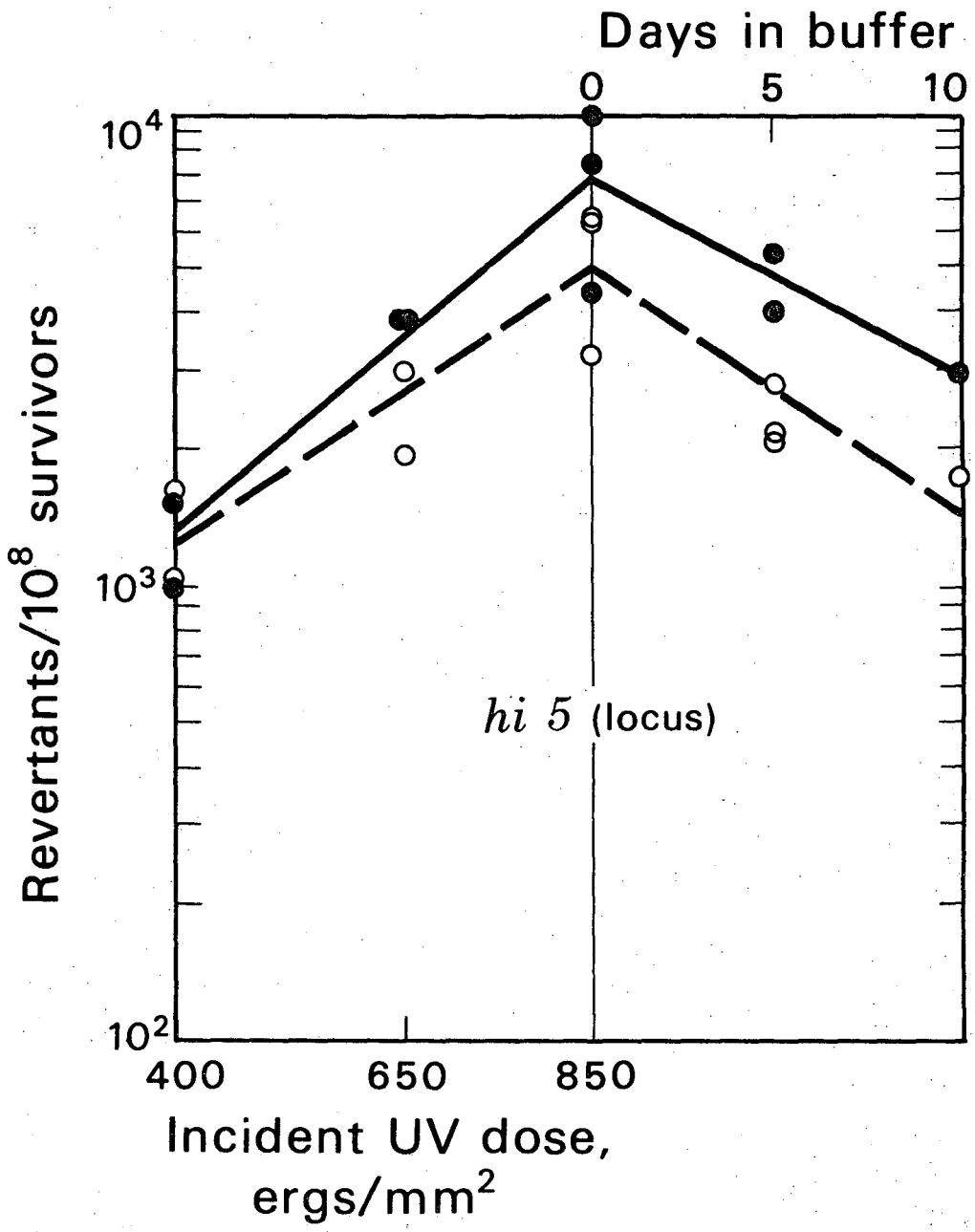
DBL 687-5344

Figure 13c. Reversion of ly 1-1 (locus) in a uvs 9-3 (circles) and a UVS (triangles) strain with (open symbols) and without (closed symbols) PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



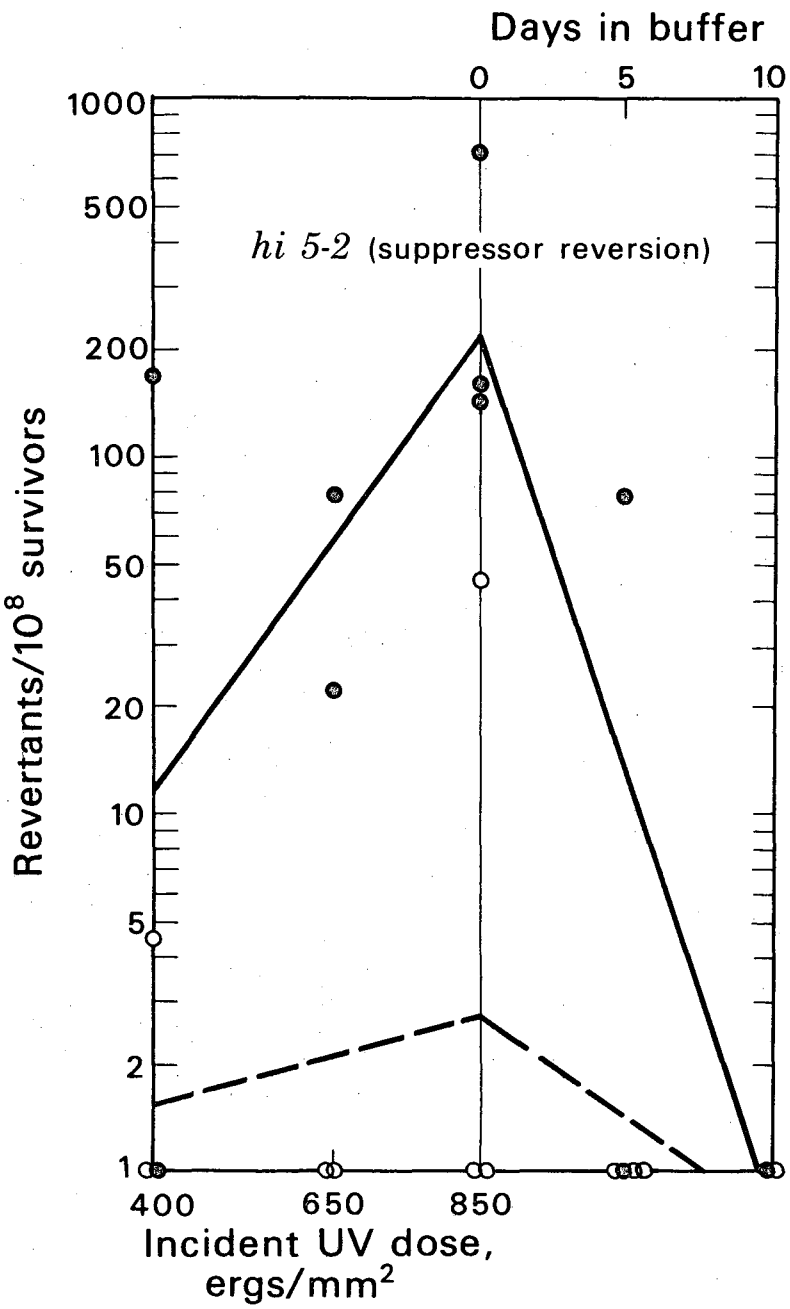
DBL 687-5347

Figure 13d. Reversion of ly 1-1 (suppressor) in a uvs 9-3 (circles) and a UVS (triangles) strain with (open symbols) and without (closed symbols) PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



DBL 687-5348

Figure 13e. Reversion of hi 5-2 (locus) in a uvs 9-3 strain with (open symbols) and without (closed symbols) PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



DBL 687-5346

Figure 13f. Reversion of hi 5-2 (suppressor) in a uvs 9-3 strain with (open symbols) and without (closed symbols) PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.

TABLE 16

Survival of uvs and UVS strains in mutation reversion studies

Strain	Incident Dose ergs/mm^2	Exp.	Days in Buffer	% Survival No PR	PR
uvs	400	6&7	0	17.5	84.9
		18		11.5	66.1
		27		20.9	76.9
	650	18		4.62	44.8
		27		4.0	72.4
	850	14		1.0	13.3
		18		0.53	33.4
		27		1.4	49.3
		33		1.37	47.6
		14	5	4.2	41.2
		18		2.2	38.9
		27		1.34	41.9
		33		(2.0)*	(35.0)*
		33	10	2.56	26.4
UVS	400	16	0	82.	100.

TABLE 16 (continued)

Strain	Incident Dose ergs/mm^2	Exp.	Days in Buffer	% Survival No PR	PR
	650	16		69.	113.
	7200	16		28.	53.
	11400	16		10.7	26.5
		25		19.4	38.9
	13800	25		15.7	29.8
	16800	25		7.13	15.9

*Estimated from Day 0 and Day 10 results.

event may differ for the various loci. For example, Clarke (15) has shown that in bacteria UV-induced suppressor revertants occur on a medium completely deficient in the required amino acid, and increases with supplements of the amino acid. Since the number of suppressor loci is large [at least 12 (51)], it is surprising that reversion at the nonsense loci is higher than reversion by suppressors.

Ultra-violet light can also induce addition-deletion mutations. Reversion at the thr 4-1 locus was comparable (10-200/10^8 survivors) to that observed for reversion of the BPS mutants. A major component of the damage that led to threonine reversion was photoreactivable (Table 15). Since approximately 60-80% of the lesions were attributable to pyrimidine dimers, it can be concluded that pyrimidine dimers can revert addition-deletion mutations. Reversion of BPS mutations by pyrimidine dimers was not detected. In the case of ly 1-1, hi 5-2, and ar 4-27 loci, reversion frequencies before and after PR were not significantly different (Table 16a). It, therefore, appears that in the uvs 9-3 strain pyrimidine dimers are not the primary cause of reversion of the BPS mutations studied. However, based on the variance of the data for these loci the results before and after PR may differ respectively by as much as 50%, 50%, and 90% and still not be detected in these experiments (Table 16a).

Unlike reversion at the hi 5-2 and ly 1-1 loci, reversion by super-suppressors was primarily due to the formation of pyrimidine dimers. The reversion by suppressors of ly 1-1 and hi 5-2 is significantly different before PR when compared to after PR. In the dose range tested the percent of suppressors due to pyrimidine dimers varied from 50%-90% for ly 1-1 and from 90%-99% for hi 5-2. Evidence presented

Table 16a

Statistical analysis of UV-induced reversion frequencies obtained with and without PR: the probability (p-value) associated with a least squares regression line of all the data (NO PR and PR) and the minimum factor of difference between treatment with and without PR that could be detected with probability .80 for cases of p >.10.

Strain	gene	p-value		minimum factor of difference	
		No liquid holding	With liquid holding	No liquid holding	With liquid holding
uvs 9-3	thr 4-1	< .01	< .01	---	---
	ar 4-27	.26	> .50	10	10
	ly 1-1 (locus)	.30	.26	2	3
	ly 1-1 (suppressor)	.05	> .50	---	6
	hi 5-2 (locus)	.23	< .01	2	---
	hi 5-2 (suppressor)	< .01	.02	---	---
UVS	thr 4-1	< .01	---	---	---
	ar 4-27	.025	---	---	---
	ly 1-1 (locus)	< .01	---	---	---
	ly 1-1 (suppressor)	.10	---	---	---

by Magni and Puglisi (77) and Magni, von Borstel, and Steinberg (80) indicates that super-suppressors arise spontaneously by addition-deletion mutations. They observed that a) there was a "meiotic effect" for reversion by super-suppressors (77, 80) and b) ICR-170, presumed to be primarily an addition-deletion mutagen (82, 140), induced super-suppressors but did not revert BPS mutations. The results in the present study are consistent with super-suppressors arising by addition-deletion events.

In the sensitive strain little of the damage that reverted the BPS mutants could be attributed to pyrimidine dimers, whereas nearly all of the lesions that caused reversion of the addition-deletion mutations and induction of suppressors were pyrimidine dimers.

For the case of forward mutations (Section V. 6.) pyrimidine dimers were a major component of the induced lesions at the tr 5 locus; however, the mutations were primarily of the BPS category in the uvs 9-3 strain. The difference in results between UV-induced tr 5 forward mutations and thr 4-1 reversion is probably due to a fundamental difference in reversion and forward mutation studies. Only a small number of lesions occurring within a locus will cause reversion while any lesion that makes a gene product nonfunctional is detected as a forward mutation.

E. Repair of Premutational Damage in a uvs 9-3 Strain

Although uvs 9 mutants are extremely sensitive to UV (Fig. 1), they still possess some repair capacity. Survival increased from 1.0% on day 0 to 2.6% on day 5 when uvs 9-3 cells were held in buffer rather than being plated immediately after UV-irradiation (Section III. D. 1.). To determine whether there was a residual repair of premutational

damage in the sensitive strain, liquid holding recovery of UV-induced mutations was examined. The results of these experiments are presented in Table 15 and Fig. 13a-f.

In all cases the frequency of reversion decreased with time in buffer after irradiation. There was residual repair of photoreactivable and nonphotoreactivable damage, although the rates of repair differed for the various loci (Table 17). The time required to decrease premutational damage by a factor of 1/e (= $D_{37\%}$) ranged from 1.75 days for suppressors of hi 5-2 to greater than 10 days for non-photoreactivable damage at the ly 1-1 locus. Similar observations have been reported for the UV-sensitive strain of E. coli. Munson and Bridges (90) have shown that the amount of premutational damage decreases with time of incubation in a minimal medium before plating. However, the time required to reduce the mutation frequency to one-half would be about 10 hours, while for yeast it is greater than 25 hours.

The repair process that reduces the frequency of mutations in the UV-sensitive strain may be the result of the uvs 9-3 mutant being slightly leaky or the presence of another intact repair system (for example, the X-ray repair system) that has the capacity to alter to some extent UV-induced damage. Even though the nature of this repair mechanism is unknown, it can remove photoreactivable and nonphotoreactivable damage at any locus with equal efficiency (Table 17). The results for reversion of the hi 5-2 locus by super-suppressors are not included in the PR part of Table 17 because the reversion frequency was close to the control level (without liquid holding).

Although there was repair of premutational damage in the uvs 9-3 strain, it was not an important factor in the reversion experiments

Table 17

Liquid holding recovery of UV-induced* permutational damage in a uvs 9-3 strain: days in buffer necessary to reduce the mutation frequency by 1/e.

Gene	Days	
	No PR	PR
thr 4-1	4.25	6.75
ar 4-27	4.25	4.75
ly 1-1 (locus)	9.0	>10.0
ly 1-1 (suppressor)	2.25	2.25
hi 5-2 (locus)	10.0	8.0
hi 5-2 (suppressor)	1.75	---

*Incident dose: 850 ergs/mm^2

discussed in the previous section since there was insufficient time between irradiation and plating for any detectable repair to occur. If the time elapsed (between irradiation and plating) was as much as one hour, only 2% of the damage would be removed for the highest rate of repair ($D_{37\%}$ = 1.75 days). Therefore, mutation induction was examined under essentially "repairless" conditions.

F. Comparison of UV-induced Reversion in a uvs 9-3 and a UVS Strain

The results of UV-induced reversion in both the uvs 9-3 and the UVS strains are shown in Fig. 14a-f and Table 18. Due to considerable lethality at high doses, it was not possible to extend the reversion studies in the uvs strain to the higher doses administered to the UVS strain. It is apparent that UV-induction of mutations is lower in the wild type than in the UV-sensitive strain for all loci examined*. Premutational damage was reduced to between one-fifth and one-tenth the amount that would be present if there was no repair. Furthermore, repair efficiently removes both photoreactivable and nonphotoreactivable damage at low doses. Thus, repair can be considered to be a conservative process in that it removes lesions without apparently generating new mutations.

At the high incident doses (≧ to 7200 ergs/mm^2) the repair system appears to become saturated. Statistical analyses of data obtained at the high doses indicated that much of the damage was attributable to pyrimidine dimers since there was a significant difference between

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*The results for reversion of hi 5-2 in the wild type strain are inconclusive.

TABLE 18 UV-induced Reversion Frequencies of Various Genes in a Wild Type Strain[1]

Gene	Exp.	Dose	PR or No PR	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$)	
								Locus	Suppressor
thr 4-1	16	0	-	0	0.30			0.0	
	25		-	1	0.35			2.9	
	16	400	NP	1	0.25			4.0	
			PR	0	0.30			0.0	
	16	650	NP	4	0.29			13.8	
			PR	3	0.35			8.6	
	16	7200	NP	147	0.083			1771.1	
			PR	90	0.16			562.5	
	16	11400	NP	123	0.033			3727.3	
	25			147	0.034			4320.7	
	16		PR	114	0.082			1390.2	
	25			91	0.068			1335.4	
	25	13800	NP	138	0.028			4925.7	
			PR	137	0.052			2631.8	
	25	16800	NP	84	0.012			6997.1	
			PR	136	0.028			4854.3	

TABLE 18 (continued)

Gene	Exp.	Dose	PR or No PR	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$)	
								Locus	Suppressor
ar 4-27	16	0	-	0	0.30			0.0	
	25		-	0	0.58			0.0	
	16	400	NP	0	0.25			0.0	
			PR	0	0.30			0.0	
	16	640	NP	0	0.21			0.0	
			PR	1	0.35			2.9	
		7200	NP	7	0.086			1.4	
			PR	4	0.16			81.4	
	16	11400	NP	3	0.033			99.9	
	25			19	0.14			134.7	
	16		PR	2	0.082			24.4	
	25			17	0.26			63.4	
	25	13800	NP	10	0.11			90.9	
			PR	11	0.21			52.4	
		16800	NP	14	0.048			291.7	
			PR	16	0.11			145.5	

TABLE 18 (continued)

Gene	Exp.	Dose	PR or No PR	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$)	
								Locus	Suppressor
ly 1-1	16		-	20	0.30	15	15	0.0	66.7
	25		-	15	0.35	26	25	2.9	40.0
	16	400	NP	24	0.25	12	9	24.0	5.3
			PR	23	0.30	9	7	17.0	-7.0
		650	NP	24	0.21	20	16	22.9	24.8
			PR	32	0.35	12	6	45.7	-21.0
		7200	NP	207	0.086	103	11	2149.9	190.4
			PR	107	0.16	107	11	956.3	39.6
	16	11400	NP	107	0.033	57	5	2958.0	217.8
	25			170	0.034	187	21	4489.0	468.1
	16		PR	187	0.082	170	21	1998.8	215.0
	25			134	0.068	134	18	1703.0	224.7
	25	13800	NP	168	0.028	93	6	5610.0	320.5
			PR	161	0.052	161	21	2689.4	363.9
	25	16300	NP	88	0.012	88	78	6497.1	793.4
			PR	179	0.028	86	80	5944.0	406.0

TABLE 18 (continued)

Gene	Exp.	Dose	PR or No PR	Total Revert-ants[2]	Viable Cells[3] ($\times 10^8$)	Revertants Tested for Suppressor	Revertants Due to Suppressor	Calculated[4] Induced Reversion Frequencies ($\times 10^{-8}$)	
								Locus	Suppressor
hi 5-2	25	0	-	-	-	-	-	(2.9)[5]	(52.2)[5]
		11400	NP	345	0.034	345	15	9703.5	389.0
			PR	381	0.068	381	7	5497.6	50.8
		13800	NP	248	0.028	248	7	8604.8	197.8
			PR	456	0.052	456	15	8478.4	236.3
		16800	NP	136	0.012	136	3	11081.0	197.8
			PR	303	0.028	303	8	10533.3	233.6

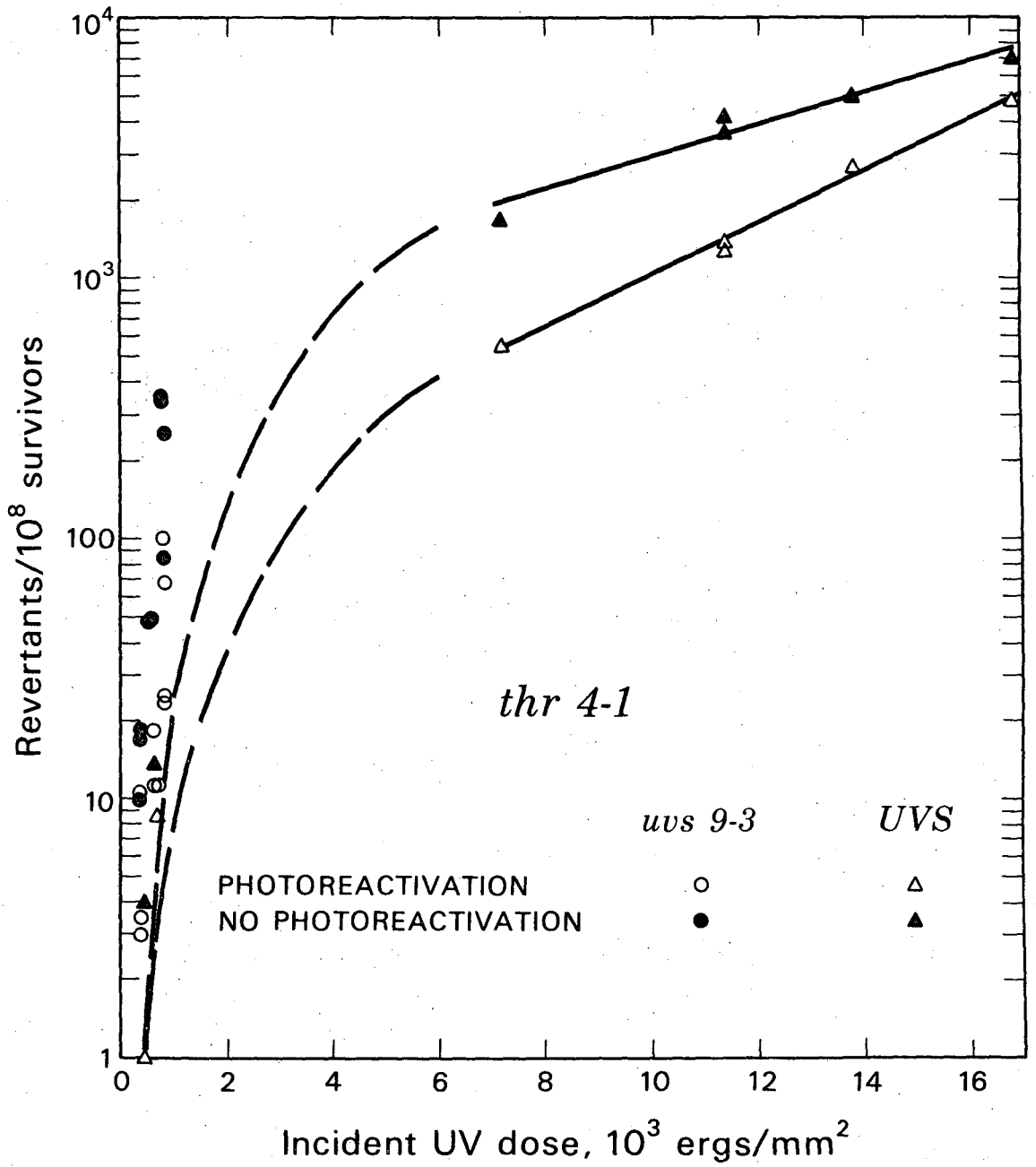
[1]Strain KC614-6-16C (α ar 4-27 le 1 ly 1-1 hi 5-2 thr 4-1 ad 2).

[2]Total revertant colonies on the corresponding omission media plates.

[3]Estimated from the number of colonies on YEPD plates and the appropriate dilution factor.

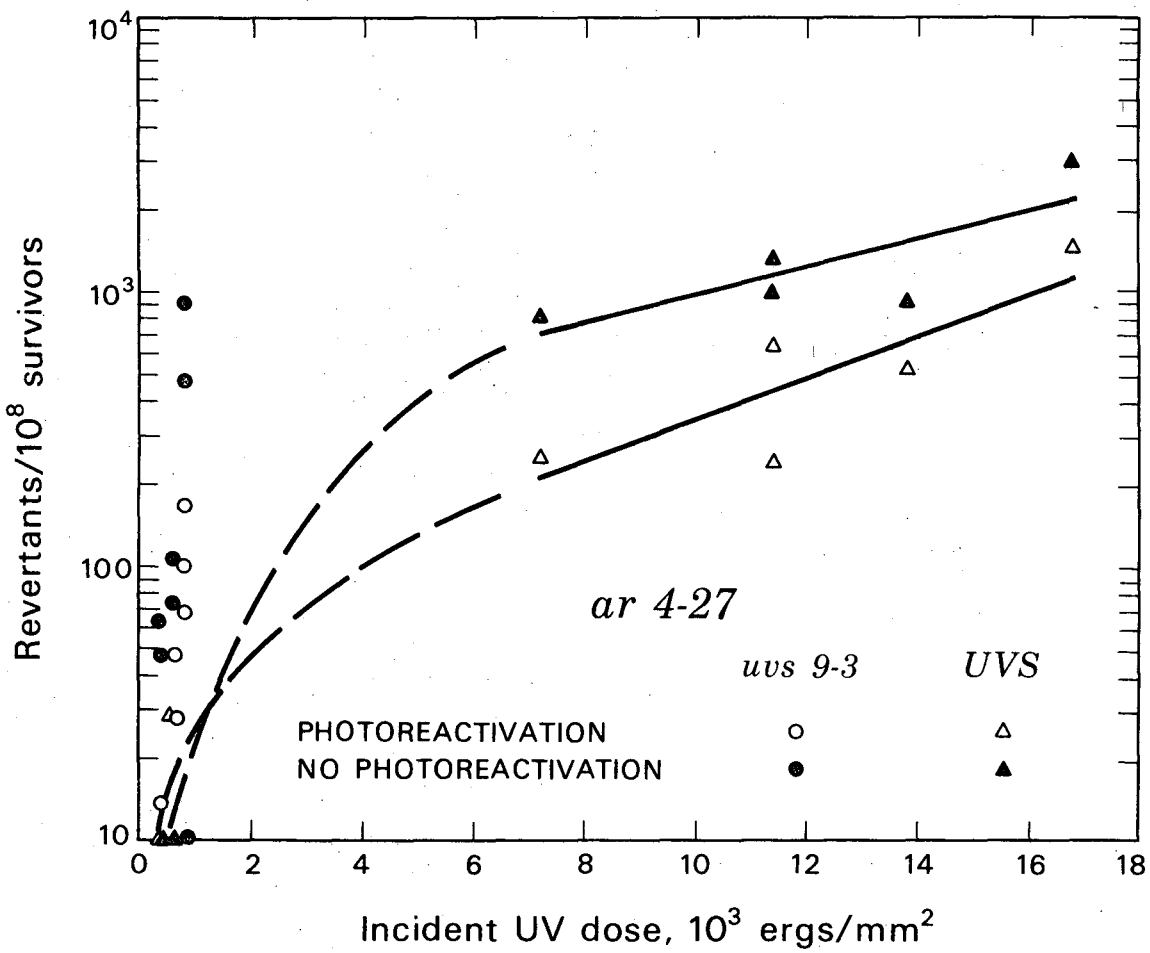
[4]Calculated induced reversion frequencies: Suppressor = (Total Revert.^{a}/Viable Cells) $\times$ (#Revert. due to Supp.^{b}/# Reverts. Tested) - Control. Locus = a $\times$ (1-b) - Control. An induced frequency that is zero or negative is assigned the value 1.0 in further calculations, e.g. for determining the regression line of log x/y vs dose. The reversion of ar and thr is considered to occur at the locus only (see text).

[5]This control value is derived from the lysine controls.



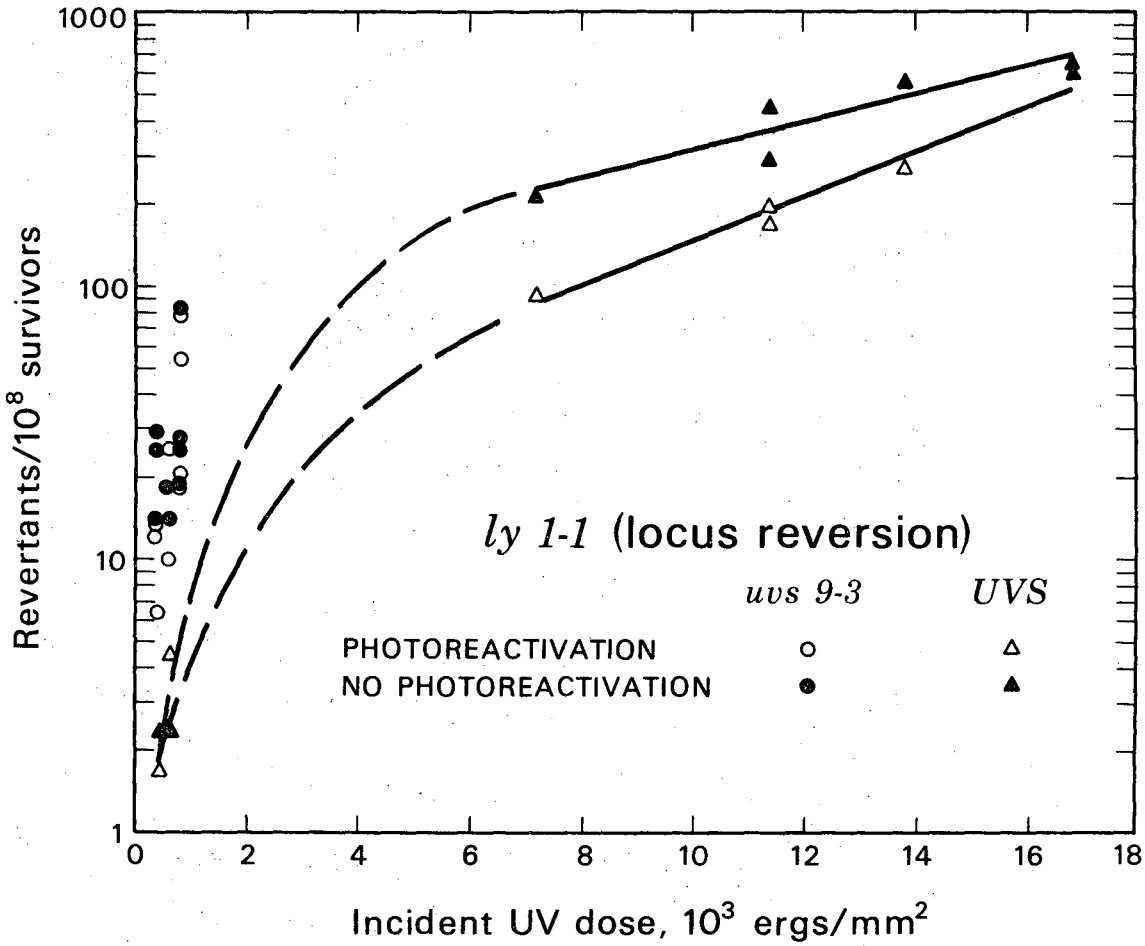
DBL 687-5350

Figure 14a. Reversion of thr 4-1 in a uvs 9-3 (circles) and a UVS (triangles) strain with and without PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



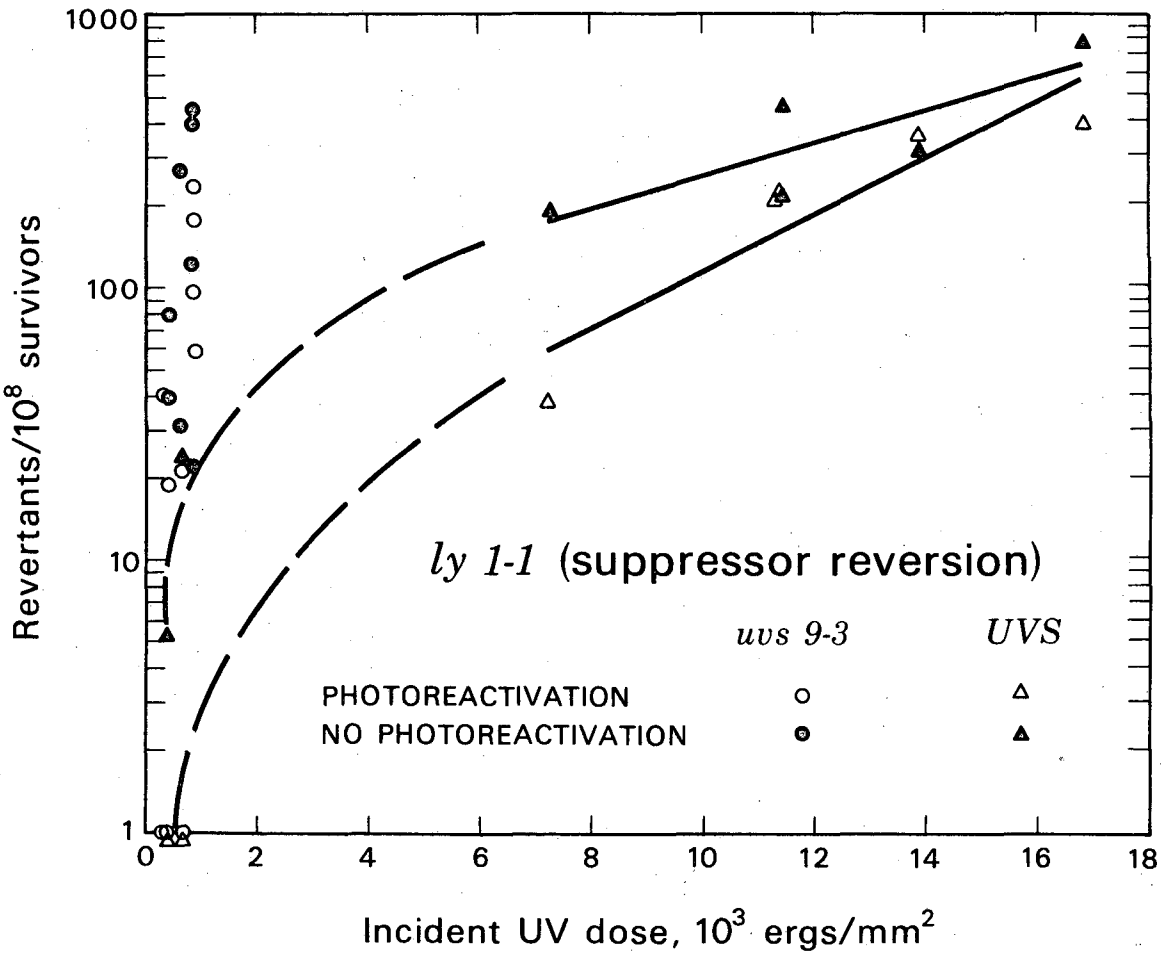
DBL 687-5351

Figure 14b. Reversion of ar 4-27 in a uvs 9-3 (circles) and a UVS (triangles) strain with and without PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



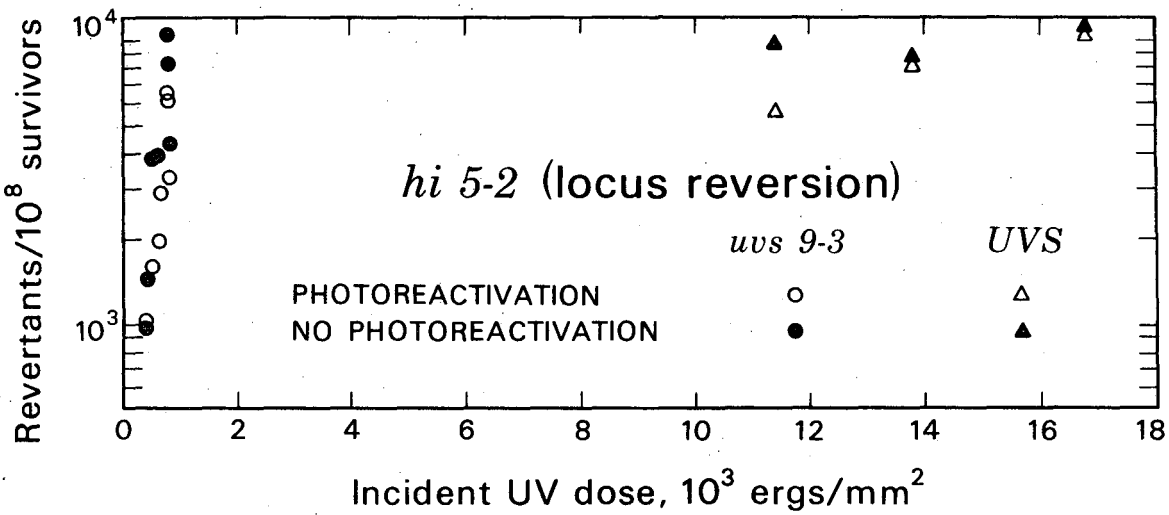
DBL 687-5353

Figure 14c. Reversion of ly 1-1 (locus) in a uvs 9-3 (circles) and a UVS (triangles) strain with and without PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



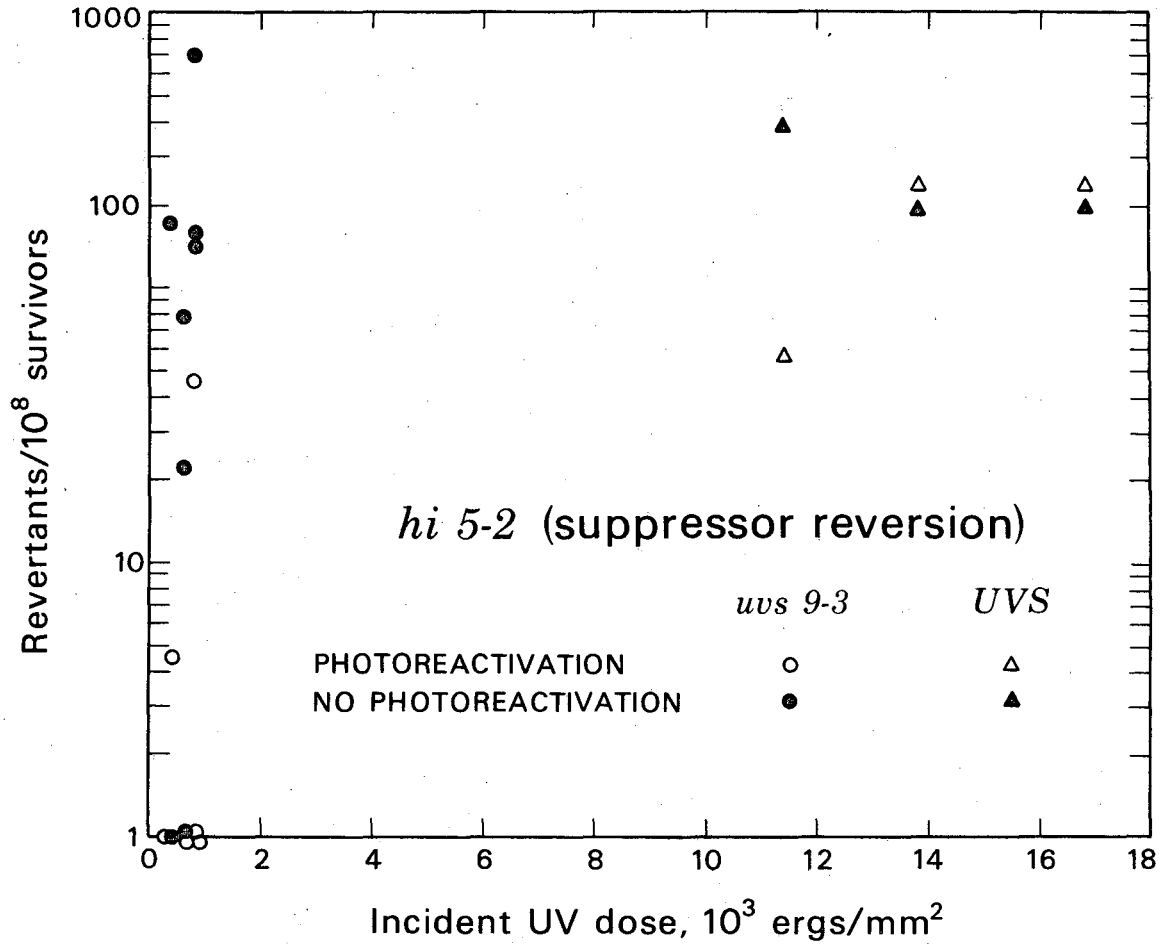
DBL 687-5352

Figure 14d. Reversion of ly 1-1 (suppressor) in a uvs 9-3 (circles) and a UVS (triangles) strain with and without PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



DBL 687-5354

Figure 14e. Reversion of hi 5-2 (locus) in a uvs 9-3 (circles) and a UVS (triangles) strain with and without PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.



DBL 687-5355

Figure 14f. Reversion of hi 5-2 (suppressor) in a uvs 9-3 (circles) and a UVS (triangles) strain with and without PR. The incident dose is 18.5 times higher than the actual dose to cells due to irradiation of cells in a concentrated suspension.

reversion before PR and after PR (Table 16a). The amount of photoreactivable damage generally decreased as the UV dose was increased. These results may reflect a change in the relative amount of pyrimidine dimer damage induced at higher doses or the production of lesions that are not subject to repair. One possibility is that the photoreactivating enzyme is saturated by lethal damage at the high doses. However, this seems unlikely since the probabilities for photoreactivation of lethal and premutational damage appear to be equal (see next section).

The present results with the wild type strain are contrary to those reported by Parry and Cox (96). Using a diploid homozygous for tr 1 they observed that the UV-induced reversion frequency reached a constant value. One-third of the damage was due to pyrimidine dimers irrespective of dose once the constant value (without PR) was attained. Their observations suggest differences between mutagenesis in haploid and diploid strains.

G. Repair of Mutational Damage Vs. Repair of Lethal Damage

As discussed by Witkin (145), induced mutation frequencies in repairless and repair sufficient strains can be compared at equal survival levels to determine whether mutational damage or lethal damage is more susceptible to repair. If lethal damage is repaired more readily, then at a given survival level the mutation frequency in a sensitive strain would be lower than in a wild type strain. On the other hand, if mutational damage was repaired more readily, the converse would be true. Photoreactivability of mutational vs. lethal damage can be examined in a similar manner as has been done by Kilbey using N. crassa (67). In Fig. 15a-f are presented reversion frequencies

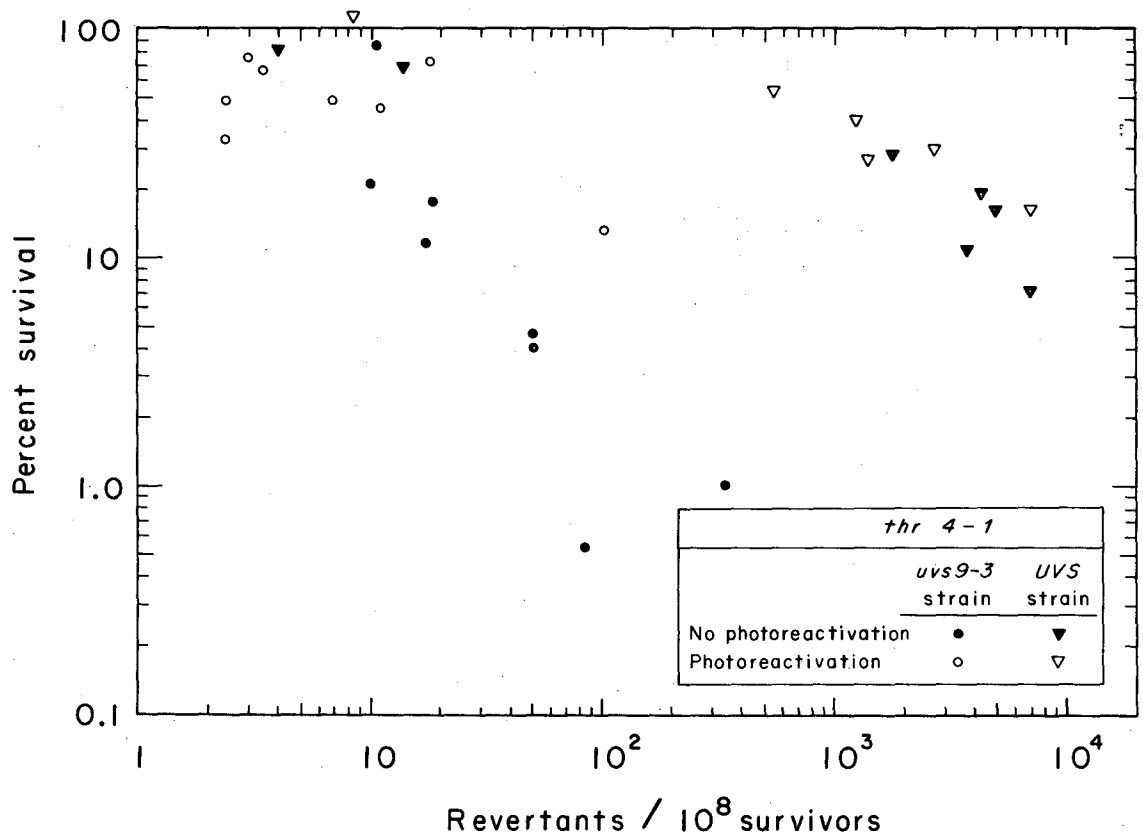


Figure 15a. Revertants/10^8 survivors versus survival for a uvs 9-3 (circles) and a UVS (triangles) strain.

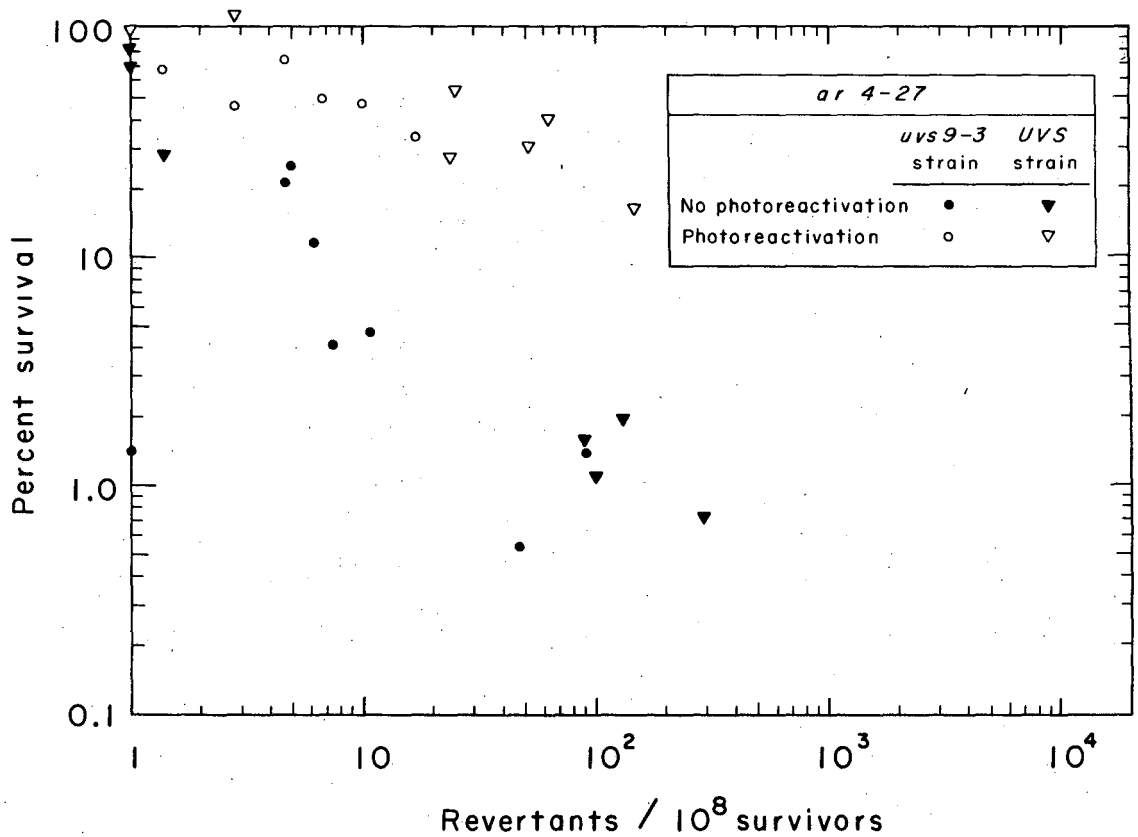


Figure 15b. Revertants/10^8 survivors versus survival for a uvs 9-3 (circles) and a UVS (triangles) strain.

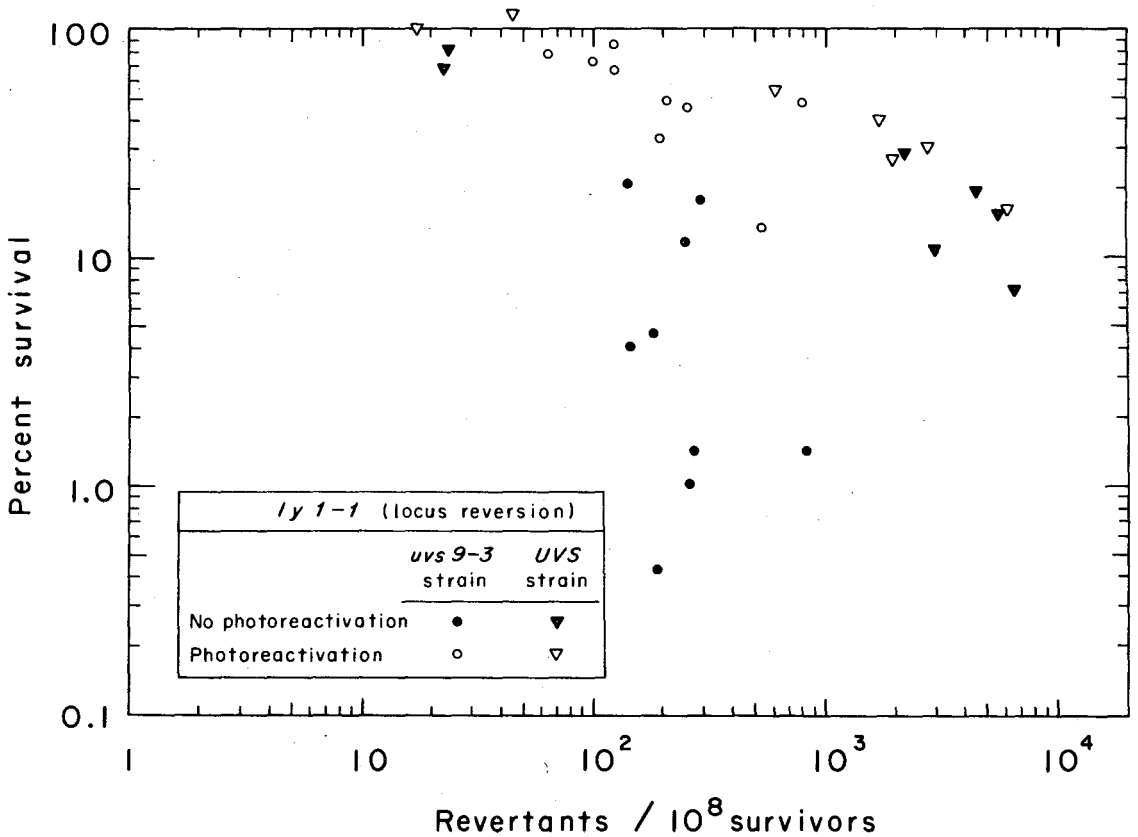


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Figure 15c. Revertants/10^8 survivors versus survival for a uvs 9-3 (circles) and a UVS (triangles) strain.

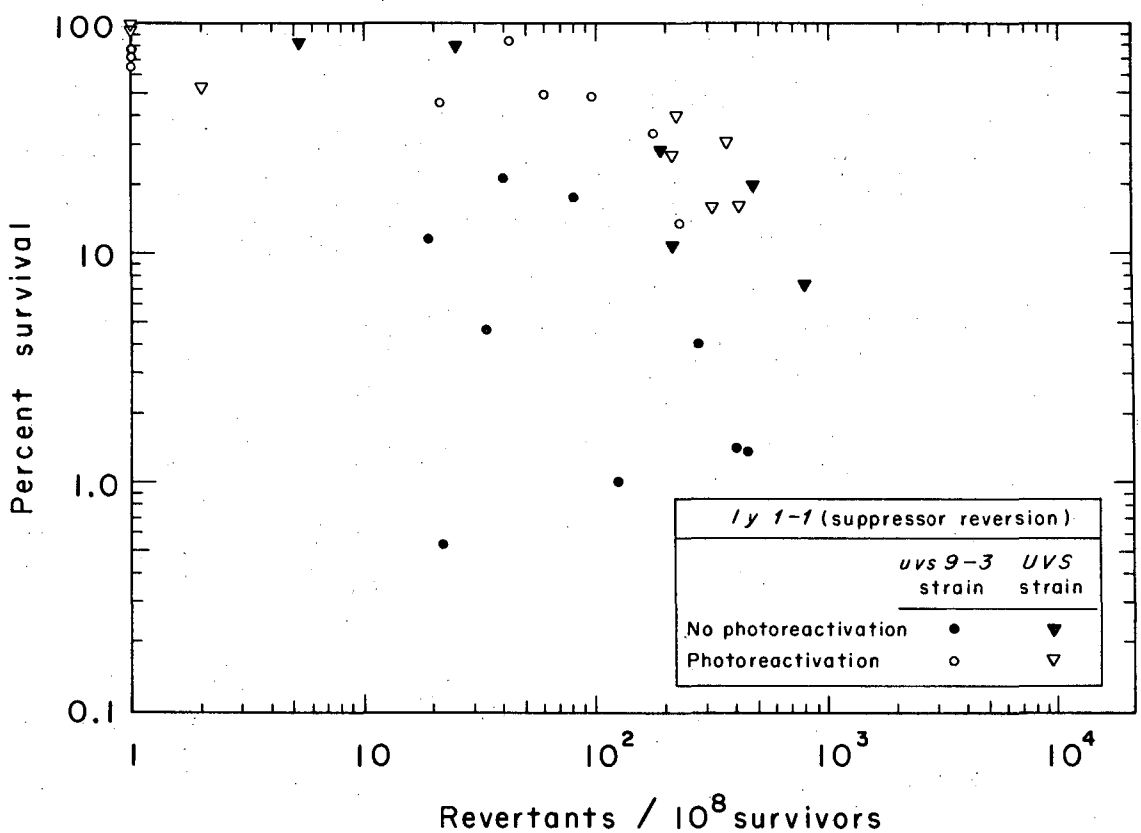
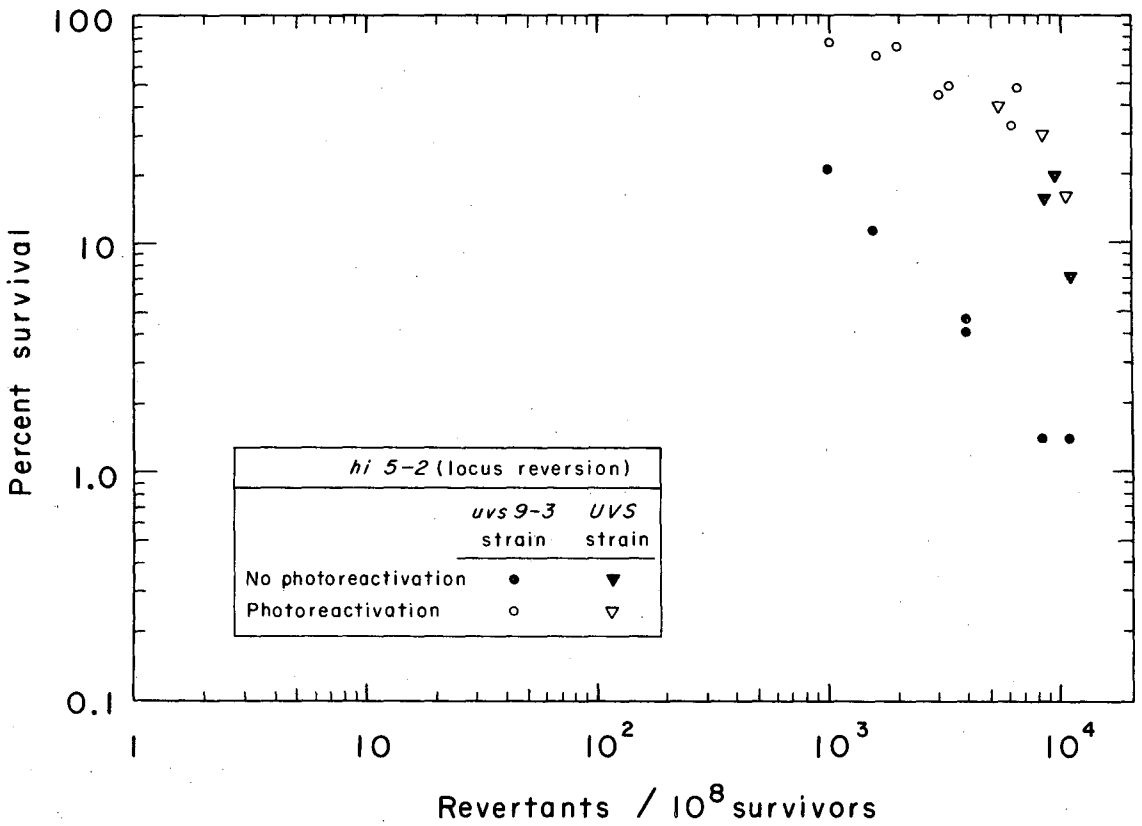


Figure 15d. Revertants/10^8 survivors versus survival for a uvs 9-3 (circles) and a UVS (triangles) strain.



XBL687-3170

Figure 15e. Revertants/10^8 survivors versus survival for a uvs 9-3 (circles) and a UVS (triangles) strain.

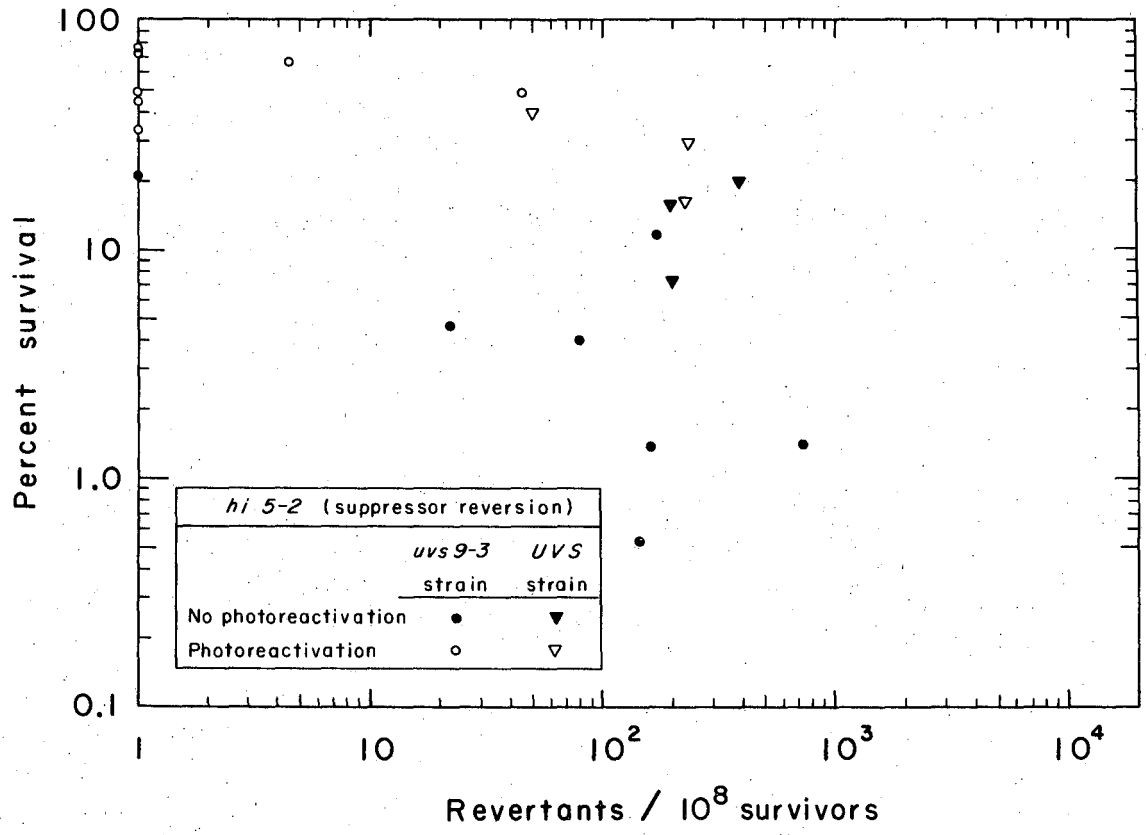


Figure 15f. Revertants/10^8 survivors versus survival for a uvs 9-3 (circles) and a UVS (triangles) strain.

plotted against survival for the various loci tested in repairless and repair sufficient strains with and without PR. Since for most loci a constant factor of difference cannot be ascertained, comparisons between strains with and without PR are made only on a qualitative basis (Table 19).

Generally photoreactivation or dark repair removes lethal damage much more readily than mutational damage. With regard to photoreactivable damage, it therefore appears as though pyrimidine dimers are more important in causing lethality than in inducing mutations in the uvs 9 strain. Since this result is consistent for all of the loci tested, it is unlikely that the results could be attributed to some regions of the genome being more susceptible to PR than others.

Unlike the results obtained with the uvs 9 strain, mutational and lethal damage are equally photoreactivable in the wild type strain except for reversion of ar 4-27. That is, curves of mutants/survivors vs. survival before and after PR are superimposable. Thus, photoreactivable damage may be equally relevant to killing and to mutagenesis in the wild type strain. Reversion of ar 4-27 may differ from the reversion of other mutations in the UVS strain for the same reasons suggested above concerning photoreactivability of mutational damage in the sensitive strain. Kilbey (67) has reported comparable results with UV-wild type strains of N. crassa. Photoreactivation of mutational damage leading to reversion of 15 mutants (representing 3 loci) was as efficient as photoreactivation of lethal damage. However, for the mutant 37401 lethal damage was photoreactivated much more readily, similar to ar 4-27 in the present study.

Comparing the results obtained between the uvs strain and the

Table 19

Relative UV-induced reversion frequencies compared at equal levels of survival for a UVS and a uvs 9-3 strain with and without PR. Results are compared against those obtained for the uvs 9-3 strain without PR.

Gene	Relative reversion frequencies: uvs 9-3		UVS	
	No PR	PR	No PR	PR
thr 4-1	1	1-10	200	200
ar 4-27	1	1-10	1	1-10
ly 1-1 locus reversion	1	1-10	10	10
ly 1-1 suppressor reversion	1	1-10	1-10	1-10
hi 5-2 locus reversion	1	10	10	10
hi 5-2 suppressor reversion	1	1-10	1-10	1-10

wild type strain, repair seems to remove lethal damage more efficiently than mutational damage for all loci except ar 4-27 reversion. Without PR the reversion of ar 4-27 was the same in the uvs as in the UVS strain. It is possible that the lesions that are involved in mutagenesis at the rest of the loci are not the same as those involved in killing. These lesions may be less subject to repair than those which cause lethality.

The results in this study are unlike those reported in E. coli by Witkin (145). It was observed that at equal levels of survival the reversion of the tryptophan requirement and the induction of streptomycin resistance in E. coli UV-sensitive strains (hcr$^-$) was higher than in resistant strains. It is, therefore, possible that the damage susceptible to repair and relevant to mutagenesis is different between yeast and bacteria.

H. Induction of Super-suppressors

The induced frequencies of different classes of super-suppressors are consistent with those reported by Gilmore and Mortimer (38). Based on the suppression pattern of the alleles tr 5-48, ar 4-17, hi 5-2, ly 1-1, ad 2-1, they identified eight classes of UV-induced super-suppressors. Only the class I (41/83) and the class II (4/83) suppressors would suppress at least two of the last three alleles. Therefore, of the total class I and class II super-suppressors, 91% belong to class I. In the present study* 95% (86/91) of the suppressors of ly 1-1 arising after irradiation of the uvs 9-3 strain belonged to class I, and for the wild type strain 99% (100/101) belonged to class I. Thus, among the two classes of suppressors, UV induces at least 90% class I suppressors whether the strain is UV-sensitive or

*Table 20.

Table 20

Frequencies of class I and class II super-suppressors* among ly 1-1 suppressor revertants

Strain	Incident Dose (ergs/mm^2)	NP or PR	Number of Suppressors	Suppressors Class I	Suppressors Class II
uvs 9-3	0	--	47	42	5
	400	NP	27	26	1
	400	PR	64	60	4
UVS	0	--	32	32	0
	400	NP	9	9	0
		PR	7	7	0
	650	NP	16	16	0
		PR	6	6	0
	7200	NP	11	11	0
	9600	NP	30	29	1
	11400	NP	4	4	0
		PR	19	19	0

*Gilmore and Mortimer (38); super-suppressors in class I suppress ly 1-1, hi 5-2, and ad 2-1, while class II suppressors affect only ly 1-1 and hi 5-2.

wild type. Within the limits of resolution in the present experiment repair of UV-induced damage at the class I and class II super-suppressor loci appears to be equally efficient.

VII. DISCUSSION

The present study has been directed to the nature of radiation repair in the yeast Saccharomyces cervisiae. Of particular interest have been questions concerning its genetic control, mechanisms of repair, damage subject to repair, repair in cells of different ploidy, repair in yeast compared to that in bacteria, and the relevance of the repair of radiation damage in evolution.

A. Radiation Sensitive Mutants

Genetic control of radiation sensitivity is complex in yeast. In the present study mutants of six genes that affect radiation sensitivity (not including phr 1) have been isolated (Table 21): uvs 9, uvs 1, xs 1, xs 2, and xs 3. Nakai and Matsumoto (91) have previously identified uvs 1 (UV_1^s) while Snow (129) had characterized uvs 9 (uvr-9). These genes were considered by these authors to be centromere-linked. In the present study they were subjected to further genetic analysis. The uvs 1 gene is approximately 19.5 centimorgans from the centromere of chromosome XVI; and uvs 9 appears to be 23 centimorgans from the centromere of a newly identified chromosome XVII (Section III. B. 3.). Other genes linked to the centromeres of chromosomes I to XVI are described by Mortimer and Hawthorne (88) and Hawthorne and Mortimer (51).

Based on genetic analyses and radiation responses of the mutants isolated in this and other studies (73, 91, 129), it is proposed that there are at least 13 genes that affect the sensitivity of yeast to

Table 21

The Genetic Control of Radiation Sensitivity in Yeast

Genes* controlling radiation sensitivity	Mutants isolated	Radiation sensitivity** UV	Radiation sensitivity** X-ray	Reference
uvs 1	UV_1^s	---	+	Nakai and Matsumoto (91)
	uvs 1-2	---	+	this study
uvs 9	uvr 9	---	+	Snow (129)
	uvs 9-2	---	+	this study
	uvs 9-3	---	+	" "
uvs 4	uvr 4	---	+	Snow (129)
uvs 8	uvr 8	--	+	"
uvs 5	uvr 5	--	+	"
uvs 11	uvr 11	-	+	"
uvs 10	uvr 10	-	+	"
uxs 1	uxs 1	--	--	this study
uxs 2	r_1^s	-	--	Laskowski, et. al. (73)
xs 1	xs 1	+	---	this study
xs 2	xs 2-1	+	--	" "
	xs 2-2	-	--	" "
xs 3	xs 3	+	-	" "
xs 4	X_1^s	-	---	Nakai and Matsumoto (91)

*A uniform system of identifying genes associated with radiation sensitivity has been employed. The letters in the gene symbol represent the sensitivity which a particular gene controls.

**Relative to other radiation sensitive mutants reported: "---" extremely sensitive; "--" intermediate sensitivity; "-" least sensitive; and "+" wild type.

X-rays and/or UV. These genes and the sensitivities of corresponding mutants as well as a uniform system for identifying the genes are summarized in Table 21. The UV_2^s (91) mutant may be allelic to other genes identified by Snow.

Similarities are apparent for the genetic control of radiation sensitivity in bacteria and yeast. Mutants have been isolated in each type of organism that are sensitive to UV or to both UV and X-rays (57). However, in yeast the uxs mutants do not display the same degree of sensitivity to UV as the most sensitive uvs mutants (uvs 9 and uvs 1). Furthermore, mutants of yeast that are sensitive only to X-rays have been identified while no such mutants have been reported for bacteria. Kato and Kondo (65) have isolated a mutant of *E. coli* that does not exhibit repair of X-irradiated phage DNA although UV damage is repaired. However, this mutant is able to repair X-ray damage induced in its own genome. It is possible, therefore, that in bacteria there is only one pathway for the repair of radiation-induced damage while in yeast there are two, one of which may be similar to the UV-repair system in bacteria (see Sections I. B. and I. C.).

B. Model for Dark Repair in Yeast

A model for dark repair in yeast can be proposed to account for the following observations: a) the existence of both uvs and xs mutants suggests more than one pathway for the repair of radiation damage, b) the identification of uxs mutants indicates common steps in these pathways, c) multilocus control of sensitivity implies more than one step in each of the pathways). The model is based on the relative sensitivities of mutants described in Table 21 and on the assumption that the product of each of the corresponding genes is

an enzyme involved in radiation repair. It is further assumed that mutants reported in this and other studies (73, 91, 129) are not leaky. The basic rules for constructing the model are discussed below.

1. Two categories of lesions are considered to be produced by a given mutagen "Y": a major component, DNA^{Y-I}, and a minor component, DNA^{Y-II}. The initial lesion in the DNA (DNA_0^{Y-I} or DNA_0^{Y-II}) is altered or removed by a series of enzymatic steps in a repair pathway, i.e., $DNA_0^{Y-I} \rightarrow DNA_1^{Y-I} \rightarrow DNA_{1-a}^{Y-I}$ ..... $\rightarrow$ DNA (repaired). The specific functions of the enzymatic steps are not considered in constructing the model. The two types of UV-induced lesions are assumed to be pyrimidine dimers (DNA_0^{U-I}) and an undefined class of damage (DNA_0^{U-II}) which could include pyrimidine hydrates, interstrand cross-links, etc.(128). The pyrimidine dimers are assumed to be the major component of UV damage based on the photoreactivability of damage induced in the various mutants that are sensitive to UV. The main types of X-ray induced lethal lesions in bacteriophage are DNA double-strand breaks and base alterations (34). If a similar situation exists in yeast for cell inactivation, one of these may be the major component of X-ray damage (DNA_0^{X-I}) and the other the minor component (DNA_0^{X-II}).

2. It is postulated that an independent repair pathway exists corresponding to each type of sensitive mutant isolated. The existence of uvs and xs mutants indicate that there are at least two repair pathways, one for UV damage and the other for X-ray damage. However, there can also be two separate pathways for the repair of damage due to any given mutagen, one for repair of the major component and one for repair of the minor component.

3. Steps in repair are assigned based on the sensitivities of the mutants isolated [extreme, intermediate, and slight (see Table 21)]. Extreme sensitivity indicates a step in the repair of DNA^{Y-I} (and possibly includes DNA^{Y-II}) that is not preceded by an alternate pathway (shunt). However, some of the major component and/or minor component may be repaired at other steps. A shunt is considered to be a less efficient pathway for repair (presented as a dashed line in a model). Intermediate sensitivity indicates either a step in the repair of DNA^{Y-I} that is preceded by a shunt or a step in the repair of DNA^{Y-II} that is not preceded by a shunt. Slight sensitivity indicates presence of steps in a shunt. The isolation of a mutant that is sensitive to more than one kind of mutagen indicates that at least one common step exists for the repair of damage caused by these mutagens. This step is a part of one pathway that may be involved with a) repair of damage shunted to it from another pathway and/or b) repair of some of the initial lesions (DNA_0) that are more efficiently repaired by another pathway.

The model is presented in Fig. 16. The various genes associated with radiation sensitivity are tentatively assigned to the steps described in the model. The critical steps are 1, 3, and 5. Since uvs 1, uvs 9, and uvr 4 are extremely UV-sensitive, these genes may be associated with step 1. Step 6 represents a shunt between the UV and the X-ray pathways. Mutants at this step would be expected to exhibit slight sensitivity to UV (e.g., uvs 10 and uvs 11) whereas mutants at step 2 would be more UV-sensitive (e.g., uvs 5 and uvs 8). Extreme X-ray sensitivity is expected for mutants at steps 3 and 5 since most of the major component of X-ray damage is repaired at

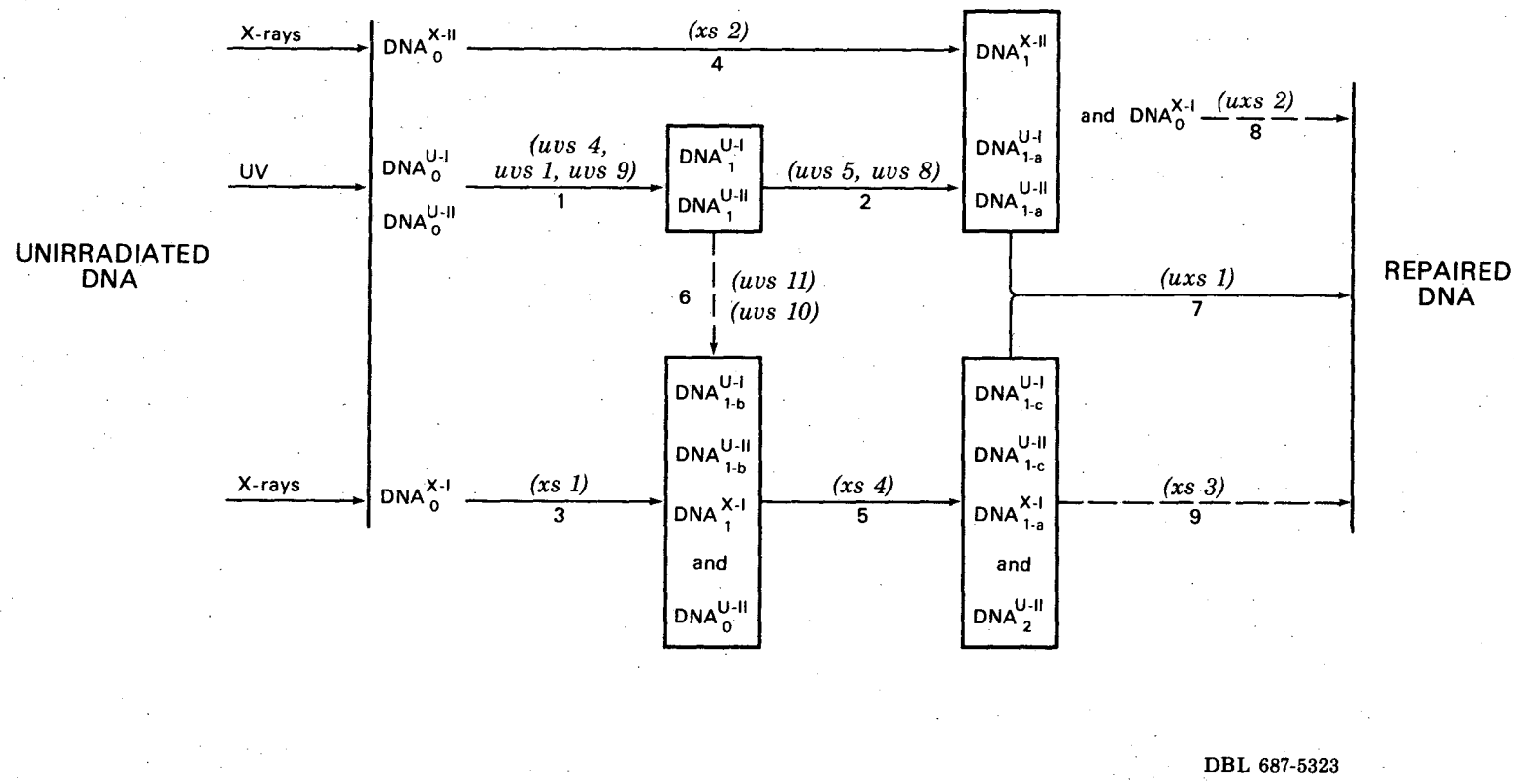


Figure 16. Proposed genetic model for the dark repair of UV and X-ray induced damage in yeast. The superscript symbols X-I and X-II indicate the major and minor components of X-ray damage, respectively. Similarly, the superscript symbols U-I and U-II indicate the major and minor components of UV damage. Subscripts refer to the altered DNA in subsequent steps of a repair pathway (e.g., 0 → 1 → 1-a, or 0 → 1 → 1-b → 1-c).

these steps. The xs 1 and xs 4 mutants, respectively, might be involved with these steps. A mutant at step 4 should exhibit only intermediate X-ray sensitivity (e.g., xs 2) since step 4 initiates repair of the minor component of X-ray damage (DNA_0^{X-II}). Following steps 2, 4, and 5, altered DNA is most efficiently handled at step 7; uxs 1 may be a mutant at this step as it exhibits intermediate UV and X-ray sensitivity. Some of the damage of the upper pathway as well as a portion of the major X-ray lesions (DNA_0^{X-II}) are repaired at step 8, while a small portion of the lower pathway alterations are repaired at step 9. The uxs 2 and xs 3 genes, respectively, may correspond to these steps. Following the steps in repair mentioned above, the DNA may be completely repaired or additional steps might be required. Based on results with interdivisional and dividing cells the X-ray repair steps for haploids are considered to function only during cell division, while in the diploid they are assumed functional during the entire cell cycle (2, and see Section III. C. 3.).

Although the model described is based on the types of radiation-sensitive mutants that have been isolated, it should accomodate and help to explain additional experimental results reported in this study and by others (73, 91, 129). The same tentative gene assignments presented in the model (Fig. 16) are employed in the following discussion.

Nakai and Matsumoto (91) reported that a UV_1^s UV_2^s haploid strain was no more sensitive to UV or X-rays than strains marked by either UV_1^s or UV_2^s alone. The previous results could be expected if UV_2^s is an allele of uvs 9. When X_1^s was combined with either UV_1^s or UV_2^s, sensitivity to UV was increased approximately by a factor of 3

while no enhanced X-ray sensitivity was observed. A mutant at step 5 (X_1^s) combined with a mutant at step 1 (uvs 9 or uvs 1) would be expected to be extremely X-ray sensitive and more UV-sensitive than a mutant at step 1 only since some of the minor component of UV damage (DNA_0^{U-II}) is repaired at step 5.

In preliminary experiments with mutants identified in this study, the presence of xs 1 with uxs 1 in a haploid leads to greater X-ray sensitivity than for xs 1 alone. Based on the tentative gene assignments in the model such a haploid is not expected to show a change in UV sensitivity when compared to a strain with only uxs 1. This possibility has not been tested. When uxs 1 was combined with xs 2-1 (step 4), sensitivity to X-rays was increased. However, the increase in sensitivity to X-rays was not to the same extent as observed for a haploid containing uxs 1 and xs 1. These results are predictable from the model if the genes correspond to the steps in Fig. 16, since xs 1 and xs 2, respectively, would be expected to initiate repair of the major and a minor component of X-ray damage.

The model is consistent with the observation that a mutant presumed to be at step 1 exhibits a smaller degree of liquid holding recovery after UV treatment (survival increased by a factor of 3, see Section III. D. 1.), than a mutant at step 8 [r_1^s, a factor of 7 to 10 increase in survival (73)]. Holding recovery of irradiated r_1^s (uxs 2) cells occurred on non-nutrient solid medium rather than in liquid medium. Laskowski, et al. (73) also tested r_1^s for holding recovery after X-irradiation. Survival after X-irradiation was greatly increased when followed by holding recovery. The dose modifying factor for homozygous diploids (r_1^s/ r_1^s) was 3 and that for a fraction of the r_1^s haploid

cell population was 2. This fraction probably corresponded to the budding cells in the irradiated population, since only budding haploid cells would be expected to exhibit any repair capacity (see Section III. C. 3.). Based on the model (Fig. 16) the X-ray holding recovery results are expected since damage repaired normally at step 8 could be repaired at step 7 (uxs 1). It can be predicted that no holding recovery would occur for a strain marked by mutants at steps 7, 8, and 5 when irradiated with either UV or X-rays.

Results reported by Snow (129) regarding nitrous acid sensitivity of uvr mutants can also be interpreted in terms of this model. The uvr-10 strain is very sensitive to nitrous acid; uvr-9 and uvr-4 are of intermediate sensitivity; while uvr-5, uvr-11 and uvr-8 are of the same sensitivity as the wild type. Therefore, the order of nitrous acid sensitivity for these mutants is different from that for UV sensitivity (Table 21). The differences in sensitivity to HNO_2 and UV may be attributed to differences in types of damage produced by these mutagens. Nitrous acid produces base damage (94) and causes interstrand cross-links in DNA (3). Therefore, the steps relevant to the repair of nitrous acid damage appear to differ from those important in UV repair. These results can be explained assuming once again the proposed gene assignments. A mutant at step 6 (uvs 10) would be most sensitive to nitrous acid treatment. Therefore, the major component of nitrous acid damage may be repaired at this step. Since a strain marked by uvr-11 is not sensitive to nitrous acid, step 6 may contain two substeps: step 6a (uvs 11) followed by 6b (uvs 10). On the basis of the sensitivity of mutants suggested as being at step 1, the minor component of nitrous acid damage would be expected to be repaired at this step.

The absence of sensitivity to nitrous acid by strains marked by uvr-5, uvr-8, or uvr-11 could be explained by assuming that either step 2 or 6 can efficiently handle the altered DNA from step 1. Furthermore, a haploid strain containing mutations at step 2 and 6b would be expected to exhibit much greater nitrous acid sensitivity than a strain marked by a mutation at 6b only.

The proposed model based on the sensitivities of various radiation-sensitive mutants is only one of the possible schemas that can describe the various steps involved in radiation repair. As is seen, such a model is convenient for explaining the present as well as earlier results (73, 91, 129) and is useful for proposing new experiments to explain the control of radiation sensitivity in yeast.

C. Photoreactivation in Yeast

The ability to photoreactivate UV-induced lethal damage in yeast has been determined (Section IV) to be under genetic control (phr 1) as had been previously reported in bacteria (47). UV-survival of a phr 1 strain was the same whether or not UV irradiation was followed by exposure to photoreactivating light. Photoreactivability in PHR 1 diploids was the same whether the strains were homozygous or heterozygous for PHR 1. As suggested in Section IV. C. 3., this may indicate a regulatory control over the synthesis of photoreactivating enzyme or that the amount of enzyme present in a cell is always in excess of the amount required for repair of photoreactivable damage. These possibilities can be tested further by determining the specific activity of photoreactivating enzyme in cell extracts of the PHR 1/phr 1 and PHR 1/PHR 1 strains. Gene dosage effects can also be assessed by determining photoreactivability in UV-irradiated tetraploid strains

with varying numbers of PHR 1 genes (0, 1, 2, 3, and 4).

Mutants of the PHR 1 gene should also be useful in determining the nature of the photoreactivating enzyme. Muhammed (89) has reported a 3000-fold enrichment of the specific activity of this enzyme over that present in the crude cell extracts. The average molecular weight of a large component of the enriched enzyme is 30,000. This component may be the pure form of the photoreactivating enzyme. To verify that this component is in fact the photoreactivating enzyme, experiments with PHR 1 and phr 1 should be performed. An absence of the 30,000 molecular weight component in the phr 1 purified preparation would confirm that this is indeed the photoreactivating enzyme. Further insight into the quaternary structure of the enzyme could be obtained by the isolation of intragenic complementing mutants at the PHR 1 locus. Intragenic complementation (Section V. B.) occurs when polypeptides corresponding to mutant alleles of the same gene interact in a diploid so as to produce a functional enzyme. This type of complementation is not possible for genes whose product is a monomeric enzyme. The isolation of phr mutants at two or more loci would indicate (a) that the photoreactivating enzyme consists of at least two different polypeptides, (b) the presence of regulatory genes, (c) or more than one step is involved in photoreactivation.

Jagger and Stafford (61) have proposed that photoreactivating light may induce indirect as well as direct photoreactivation. Indirect photoreactivation presumably results from a delay (63) in cell division thus allowing more time for dark repair to occur. The delay may be induced by photoreactivating light in a manner similar to that observed for photoprotection (62) although in photoprotection, light is

administered prior rather than after irradiation. As mentioned above UV-survival in a phr 1 strain is the same whether or not UV irradiation is followed by exposure to photoreactivating light. This observation was made for phr 1 uvs 9-3 and phr 1 UVS 9 strains. Thus, it is concluded that for the light source employed in the present experiments indirect photoreactivation did not occur.

D. UV Mutagenesis and the Role of Repair

It was found that a mutation (uvs 9-3) that led to greatly increased UV sensitivity (DMF: >20) also affected sensitivity to UV-induced mutation.

1. Induction of Forward Mutations

The induction of forward mutations at the tr loci differed between a UV-sensitive and a wild type strain (Section V. D.). In the sensitive strain (uvs 9-3) nearly all the mutations were attributable to the presence of pyrimidine dimers while at comparable survival levels for the resistant strain the tr mutations resulted from damage other than pyrimidine dimers. As suggested in the following discussion, the lack of photoreactivable damage at the tr loci was probably due to the efficient removal of pyrimidine dimers by the dark repair process. Fifty-three percent (Table 10) of the damage that resulted in the production of unclassified mutants in the wild type strain was photoreactivable. Thus, pyrimidine dimers contributed appreciably to the production of these mutants. Since photoreactivable lesions can produce forward mutations in a UVS strain and none of the tr mutants was due to this type of damage, it appears that dark repair efficiently removes pyrimidine dimers at these loci (at least more efficiently than for the removal of non-pyrimidine dimer damage). Pyrimidine dimers are

presumably induced at the tr loci in the UVS strain since in the UV-sensitive strain nearly all the mutational damage at these loci was due to pyrimidine dimers. As discussed in Section V. D. 2., repair may occur more efficiently in some regions of the genome than on others.

Pyrimidine dimer damage at the tr 5 locus in the uvs strain produced primarily base-pair substitution mutations (at least 75%). Similarly at least 84% of the tr 5 mutants in the wild type strain were produced by base-pair substitution events, although the lesions involved were not pyrimidine dimers. Therefore, UV light (2537 Å) is considered to be primarily a base-pair substitution mutagen with regard to the induction of mutations at the tr 5 locus. Results reported by Manney (83) are in agreement with this view. Of 31 tr 5 mutants isolated by him in a UVS strain only 5 were neither suppressible nor complementing. Therefore, based on criteria employed in the present study, at least 84% (26/31) of the mutants characterized by Manney were due to base-pair substitutions.

Similar to the UV-induction of unclassified mutants in the present study, Kilbey and de Serres (68) have reported that in a wild type strain of N. crassa UV-induced forward mutants at the ad-3A and ad-3B genes are markedly reduced by PR (DMF: 1.4 to 1.7). They have found that although photoreactivation reduces the mutation frequency it does not change the types of mutants produced. Approximately 50% of the mutants were complementing whether or not UV was followed by PR. Within the complementing groups of mutants, the patterns of intragenic complementation before and after PR were not distinguishable. These results with N. crassa support the hypothesis that photoreactivable and non-photoreactivable lesions cause the same types of mutational

damage. However, in the study by Kilbey and de Serres only 35% of the mutants, with and without PR, can be attributed to having originated from base-pair substitution events. This value is a minimum estimate based on the number of mutants that exhibited nonpolarized complementation.

Direct evidence for the nature of UV-induced mutational lesions has been reported by Drake (25, 26, 27). He irradiated T4 bacteriophage prior to and after infection of E. coli (25, 27), and in both cases the number of base-pair substitution mutations approximately equaled the number of addition-deletion mutations in the rII region. The different mutants were classified by their revertibility in the presence of specific mutagens. Most of the base-pair substitution mutations were considered to be due to GC → AT transitions (25). Mutation induction was also examined with and without PR (26). Base-pair substitution mutations and addition-deletion mutations were approximately equally reduced by PR (60% and 55%, respectively). These results before and after PR appear to be similar to those obtained with yeast in that photoreactivable and nonphotoreactivable damage in yeast leads to the same relative frequency of base-pair substitution mutations at the tr 5 locus.

2. Reversion of Mutations

The presence of a repair system in yeast enables the removal of 80-90% of the UV-induced damage that would normally cause mutation in the uvs 9-3 strain. These results are similar to those reported by Witkin (144) for bacteria, who observed that mutation induction at several loci studied was decreased by a factor of at least 10 when repair was present. Comparisons of UV-induced mutation in

UV-sensitive and wild type strains of N. crassa and A. nidulans does not yield such clear cut distinctions. Chang, Lennox, and Tuveson (12) have reported that in a uvs-1 mutant strain of A. nidulans mutation induction frequencies ranged from less than to greater than those obtained for the wild type strain at equal doses. They also reported that at equal doses mutation induction in the uvs-1 strain of N. crassa was lower than in the wild type strain. It is possible that the product of the UVS-1 genes in these species is not involved with the type of UV-repair suggested for bacteria and yeast since the uvs-1 mutants were only 1.5 (A. nidulans) and 3-4 (N. crassa) times as sensitive to UV as the corresponding wild type strains.

In the present study the percent photoreactivable damage induced at the various loci in the UV-sensitive strain varied from an undetectable amount to as much as 99%. It appeared that premutational damage leading to addition-deletion events was primarily photoreactivable (at least 60%) whereas damage causing base-pair substitution events was not. If there is a general correlation between the type of mutation and photoreactivability of damage causing reversion of the mutation, then future experiments to identify various types of mutations would be greatly simplified. The above effect was observed with the sensitive strain only. In the wild type strain UV-induced reversion of all requirements was photoreactivable (the results with hi 5-2 were inconclusive) at comparable levels of survival.

The susceptibility to reversion of like mutations was also found to differ between the UVS and the uvs 9-3 strains. In the wild-type strain reversion frequencies at the hi 5-2 and the ly 1-1 loci were within a factor of 2 to 3 (Fig. 14c and 14e), whereas in the sensitive

strain reversion at the hi 5-2 locus was approximately 10 to 25 times higher (Fig. 13c and 13e). As discussed in Section VI. B., both of these are nonsense mutations of the ochre category. Consequently, for each of these mutations the same codon has to be altered for reversion to wild type to occur. These results may be due to the types of mutagenic events produced at low and high doses. It is possible that at low UV doses the lesions that cause reversion of BPS mutations result primarily in transitions (e.g., GC → AT) rather than in transversions (e.g., GC → CG), while at high doses the lesions remaining after repair may produce either with similar efficiency. If reversion of ly 1-1 could occur only by transversions due to a restriction on the codons which will lead to reversion and reversion of hi 5-2 can occur by either transitions on transversions, then the previous results would be expected. This hypothesis could be tested by sequency analysis of the polypeptide products after reversion in a sensitive and a resistant strain and determining the nature of the reversional event.

As shown in Fig. 13c to 13f and 14c to 14f, reversion by the induction of suppressors was always less than reversion at the locus. this is contrary to what is found in bacteria. Induced reversion of suppressible alleles in E. coli is considered to be primarily due to suppressor induction (143). The E. coli WP2 (try$^-$) mutant studied by Hill (54) and Witkin (144) has been identified as a nonsense mutant of the ochre category (95). In independent studies by Bridges, Dennis, and Munson (8) nearly all UV-induced try$^-$ revertants were due to the presence of a suppressor. The differences observed between bacteria and yeast may be the result of differences in composition of the

omission media employed. In the present reversion experiments irradiated cells were plated on media devoid of the required amino acid. However, in experiments with bacteria, cells were generally plated to medium supplemented with a small amount of the required amino acid. This procedure leads to higher frequencies of reversion to prototrophy (142). Witkin (144, 145) suggests that damage at the suppressor loci is less likely to be repaired if there is active protein synthesis after UV-irradiation which would be the case if there were a supplement of the required amino acid in the medium. Possibly damage at the suppressor loci can compete less effectively for repair enzymes under these conditions (144) or expression of suppressor reversion events requires protein synthesis (15).

The frequency of UV-induced reversion by suppressors might also be increased in yeast if the omission medium were supplemented with small amounts of the required amino acid. If not, it would indicate that the process of suppressor induction in yeast is indeed different from that in bacteria.

E. The Relevance of Dark Repair and Photoreactivation in Evolution

In this study it has been shown that dark repair processes in yeast are important in maintaining viability and conserving genetic information when cells are irradiated with UV and ionizing radiation. The evolutionary significance is apparent since dark repair removes not only UV and X-ray damage but also lesions due to chemical mutagens (52, 57, 113, 129, 135). Cells capable of dark repair would have a selective advantage over those with no capacity for dark repair when exposed to these agents.

The evolutionary importance of repair pertains to induced mutations and lethality as well as to the process of spontaneous recombination. Some mutants of E. coli that lack the ability to repair UV-induced damage do not exhibit recombination (14, 58). Similarly UV-sensitive mutants of Ustilago maydis have been reported by Holliday (55) that also show less recombination than in wild type strains. Some radiation-sensitive strains isolated in the present study also may prove to be recombinationless.

As mentioned earlier (Section VII. A.) the identification of X-ray sensitive mutants in yeast indicates that a pathway for the repair of X-ray damage appeared during the evolutionary transition from prokaryotic to eukaryotic organisms. This being true, then there would be a selective advantage to cells having an X-ray repair mechanism in addition to a UV-X-ray repair system. Based on the model discussed in Section VII. B., X-ray damage would be more readily repaired by the X-ray repair pathway. There would also be a selective-advantage if the X-ray repair system was more efficient than the UV-X-ray repair pathway in the removal of lesions produced by other mutagens. To test the latter proposal, survival of xs, uxs, and uvs mutants after exposure to a variety of mutagens needs to be examined.

The selective advantage of cells capable of photoreactivating UV-induced damage was probably very great during early evolution due to the presence of far UV at the earth's surface (108). Although far UV is now absorbed by ozone in the upper atmosphere (109), the presence of a photoreactivating system in a wide range of organisms has been demonstrated (17, 60). It is possible that the photoreactivation mechanism is a vestige of evolution or that there is still a selective

advantage for organisms possessing it. For example, Rupert (105) has shown that damage induced in transforming DNA by solar radiation is photoreactivable (DMF: 3 to 4). Therefore, it appears that solar radiation can induce *in vitro* lesions that are presumably pyrimidine dimers.

VIII. SUMMARY

Eight radiation-sensitive mutants of *Saccharomyces cerevisiae* were isolated: three were sensitive to ultraviolet light (UV); four were sensitive to X-rays, and one was sensitive to UV and X-rays. Allelism was demonstrated between two of the UV-sensitive mutants and also between two of the X-ray-sensitive mutants. The UV-sensitivity genes are centromere-linked. One of them is 19.5 centimorgans from the centromere of chromosome XVI and the other 23 centimorgans from the centromere of a newly identified chromosome XVII. None of the mutants was suppressible. Some of the mutations prevented sporulation. Sensitivities and genetics of these mutations were compared to those reported by other investigators. It is concluded that there are at least 13 genes in yeast that affect sensitivity to radiation. Based on these comparisons and experiments, a model is proposed for the repair of radiation damage.

The UV-sensitive mutant, *uvs 9-3*, was tested for liquid holding recovery; increased survival was observed when held in buffer after UV-irradiation. However, there was no change in the amount of lethal damage that could be photoreactivated. This procedure is suggested as a means for delimiting the altered step in repair. When cells were incubated in nutrient medium after being held in buffer, photoreactivability decreased exponentially with time of incubation. The decrease is attributed to the onset of DNA synthesis.

A "photoreactivationless" mutant was identified. The *phr 1* mutation prevented photoreactivation (PR) in UV-sensitive and wild type haploid and diploid strains. Photoreactivability was the same whether a *PHR* diploid was *PHR 1/phr 1* or *PHR 1/PHR 1*. The absence of a gene dosage

effect is discussed and further experiments are suggested.

Forward mutation induction was examined in a UV-sensitive strain (uvs 9-3) and a wild type strain. Mutants at the tr 5 locus were classified according to ability to complement, suppressibility and osmotic remediability. A mutant exhibiting any one of these characteristics was considered to have arisen by a base-pair substitution event. In the uvs 9-3 strain out of 42,233 colonies, 16 tr 5 mutants were isolated without PR, while none were isolated after PR (0/23,541 colonies). Twelve of the mutants were attributed to base-pair substitution events; thus, pyrimidine dimers were concluded to cause primarily base-pair substitution mutations at this locus.

Since the mutation frequency at the tr 5 locus in the UVS strain was the same before and after PR, the lesions causing the mutations were considered to be those other than pyrimidine dimers. However, this damage also produced mainly base-pair substitution mutations (16/19).

The production of whole colony and sectored mutants for several loci was also examined in the uvs 9-3 and the UVS strains. It is proposed that whole colony mutants arise by dark repair acting in some way on the DNA strand opposite the mutational lesion.

For the UV-sensitive and wild type strains, induced reversion to prototrophy by UV occurred regardless of whether the original auxotrophic mutation arose by a base-pair substitution (missence or nonsense category) or by an addition-deletion event. In all cases tested a significant amount of reversional lesions were due to pyrimidine dimers being induced in the UVS strain, while in the sensitive strain (uvs 9-3) pyrimidine dimer damage could be detected only when reversion occurred by addition-deletion events. At equal doses, UV-induced reversion

frequencies in the UVS strain were always less than in the uvs 9-3 strain. It was, therefore, concluded that repair is a conservative process since it removes lesions in the DNA without affecting the original informational content.

A comparison of dark repair and photoreactivation of lethal and mutational damage indicated that lethal lesions are generally repaired more readily than mutational lesions by dark repair. Similarly, photoreactivation removes lethal damage in the uvs strain more efficiently than mutational damage; however, in the wild type strain both types of damage are removed with equal efficiency.

The relevance of repair in evolution is discussed. It is suggested that there may still be a selective-advantage for those organisms possessing a photoreactivation system even though there is no exposure to far UV (< 2900 Å) at the earth's surface.

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