UR-3490-1790

STUDIES OF DNA REPAIR IN *SACCHAROMYCES CEREVISIAE*

I. CHARACTERIZATION OF A NEW ALLELE OF *RAD6*

II. INVESTIGATION OF EVENTS IN THE FIRST CELL CYCLE AFTER DNA DAMAGE

by

Jean Ann Douthwright-Fasse

Submitted in Partial Fulfillment

of the

Requirements for the Degree

DOCTOR OF PHILOSOPHY

Supervised by

Dr. Christopher W. Lawrence

Department of Radiation Biology and Biophysics

School of Medicine and Dentistry

The University of Rochester

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DISCLAIMER

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VITAE

The author was born on [redacted]. She entered Skidmore College in 1968 and graduated with a Bachelor of Arts degree in Biology-Chemistry in 1972. She received a Master of Science degree in Biophysics under the supervision of Dr. Wallace Snipes at The Pennsylvania State University in 1973. Her doctoral work in the Department of Radiation Biology and Biophysics at the University of Rochester has been supervised by Dr. Christopher W. Lawrence.

ACKNOWLEDGEMENTS

To Dr. Christopher W. Lawrence for his guidance, rigorous training and instruction.

To the members of my committee Drs. Betsy Ohlsson-Wilhelm, T. Daniel Griffiths and Louise Prakash for their helpful discussions and advice.

To Dr. William Farland for carrying out the photoreactivation enzyme assay.

To Dr. Roshan Christensen for her encouragement, perspective, and enthusiasm in the ways of science.

To Noelle Yuen for her technical assistance.

ABSTRACT

Studies in two independent, but related, areas of DNA repair have been carried out in the eucaryotic yeast, Saccharomyces cerevisiae. The first is the characterization of a new allele in the RAD6 gene suggesting that the gene is multifunctional. The second is the utilization of photoreactivation as a probe of events occurring during the first cell cycle after DNA damage.

Strains carrying the new allele, designated rad6-4, of the RAD6 locus are about as sensitive to UV and ionizing radiation as those carrying rad6-1 or rad6-3 but, unlike them, are capable of induced mutagenesis and sporulation. Diploids homozygous for rad6-4 or heteroallelic for rad6-4 and either rad6-1 or rad6-3 allow UV induced reversion of the ochre alleles arg4-17 and cyc1-9, of the proline missense allele cyc1-115, and of the frameshift allele cyc1-239. They also permit EMS induced reversion of cyc1-115. The frequency of reversion is greater than wild-type in each case. Homozygous rad6-4 diploids sporulate as well as wild-type. Although rad6-4 may well be a missense mutation, our evidence shows that it is unlikely that this phenotype is due to leakiness. Instead, the data suggest that the RAD6 gene is multifunctional. One function is necessary to recover from DNA damage in an error-free manner, and the other is concerned with mutagenic processes and sporulation. Rad6-1 and rad6-3 strains are deficient in both of these functions, while rad6-4 strains are deficient only in the error-free function.

The loss of photoreversibility (LOP) of ultraviolet induced mutations to arginine independence in an excision defective strain carrying arg4-17 examines the events occurring in the first cell cycle after DNA damage. LOP is dependent upon de novo protein synthesis. The post UV protein synthesis causes pyrimidine dimers to become inaccessible to the photoreactivating enzyme in some unknown manner. LOP begins immediately after UV irradiation, before semiconservative DNA synthesis takes place, and is complete after four hours in growth medium. There is no evidence indicating whether the normal function of the protein is involved in excision repair, or in one of the two repair processes believed to be inducible; induced mutagenesis or recombinational repair.

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INTRODUCTION

All living systems appear to have complex DNA repair processes which respond to damage in the genetic material and involve molecular mechanisms such as DNA synthesis, recombination and mutagenesis. The existence of these repair processes is not surprising since there are a large variety of chemical and physical agents in the environment, such as UV light which damage DNA. This introduction will deal with repair processes in E. coli, a well characterized procaryotic organism, and in the eucaryotic yeast Saccharomyces cerevisiae, especially the relation between repair processes and the RAD6 gene function in yeast.

A. DNA repair in E. coli.

In E. coli the best characterized repair processes are those which act on pyrimidine dimers produced in the DNA by ultraviolet light. Pyrimidine dimers are formed by a covalent linking of adjacent pyrimidines in a DNA strand through the 5 and 6 carbons to form a cyclobutane ring. A region of local denaturation and distortion of the secondary helical structure of the DNA is caused by pyrimidine dimers since the covalently linked bases are unable to form hydrogen bonds with bases in the complementary strand. Dimers pose a temporary block to the normal processes of replication and transcription, and are thought to be responsible for 90% of the induced mutations after irradiation (Hanawalt, 1975).

Wild type strains of E. coli effectively neutralize the potentially lethal effects of pyrimidine dimers by utilizing one or more of three types of enzymatic DNA repair processes. The most specific and simplest mechanism is enzymatic photoreactivation. The photoreactivating enzyme which has been extensively purified from E. coli (Sutherland, Chamberlain and Sutherland, 1973), recognizes and binds to the region of DNA containing a pyrimidine dimer. This occurs in the dark, and upon illumination of the enzyme-DNA complex with photoreactivating light at wavelengths between 310 to 400 nm, the dimer is split or monomerized in situ and the photoreactivating enzyme is released (Setlow, 1966). Since the photoreactivating enzyme acts specifically on dimers and photoreactivating light can be applied easily, photoreversibility of specific sites in the genomes of various procaryotic organisms has been used as a probe of events occurring immediately after UV irradiation (Witkin, 1963; Nishioka and Doudney, 1970; Doubleday, Bridges, and Green, 1975). Enzymatic photoreversal of pyrimidine dimers does not cause changes in the DNA base sequence of photorepaired regions and DNA replication can proceed normally as soon as the dimer is split.

In the dark, pyrimidine dimers are not enzymatically reversed, but physically removed from the DNA by a multienzymatic mechanism, excision repair (Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964). The product of the uvrA and uvrB genes of E. coli,

(Seeberg, 1978), makes a single strand nick or incision on the 5' side of the dimer. This is followed by an exonucleolytic excision releasing an oligonucleotide containing the pyrimidine dimer and some bases creating an excision gap. Repair replication using the region of the strand opposite the gap as a template for synthesis of the missing nucleotides is carried out by DNA polymerase I (Cooper and Hannawalt, 1972; Glickman, 1974). The final step is sealing of the sugar-phosphate linkage by polynucleotide ligase. Efficient excision requires the presence of additional gene products, urvC (Howard-Flanders and Boyce, 1966) uvrE (Sinzinis, Smirnov, and Saenko, 1973) and mfd (George and Witkin, 1974; 1975), but the role of these gene products is not known. In mutants lacking the activity of DNA polymerase I, it has been demonstrated that excision repair utilizes DNA polymerase II (Masker, Hanawalt, and Shizuya, 1973) or DNA polymerase III (Youngs and Smith, 1973). In addition to this "short patch" type of excision repair explained above, a "long patch" pathway has been identified which requires the products of the recA$^+$ and lexA$^+$ gene products and occurs only in growth media (Cooper and Hanawalt, 1972).

The final cluster of repair activities functions after DNA replication bypasses DNA damage (Rupp and Howard-Flanders, 1968), and can act in two ways. The first is relatively nonmutagenic and involves a recombinational exchange, while the other is error prone and inducible. Both are dependent on functional recA$^+$ gene

products. The phenotype of the recA$^-$ mutant strains is highly pleiotropic and these mutant strains lack many other wild type functions in addition to the ability to recombine, as will be discussed later. When pyrimidine dimers are present in the template strand, daughter strands are initially detected as relatively low molecular weight DNA with gaps of about 1000 nucleotides (Iyer and Rupp, 1971) which correspond in number and position to the dimers in the parental strand (Howard-Flanders et al., 1968). These daughter strand gaps are secondary UV lesions and post-replication repair connects the daughter strand segments by recombination with the parental strand allowing further replication of the DNA. In excision defective mutant strains, uvr$^-$, the major repair mechanism is post-replication repair although post-replication repair does occur in excision proficient strains. DNA replication after UV irradiation is followed by detecting parental DNA in the daughter strands. The mechanism for recombinational exchanges is not known, but the recA$^+$ gene product is required (Smith and Meun, 1970) and the process is nonmutagenic.

The second repair process after DNA synthesis bypasses DNA damage is dependent on the recA$^+$ and lexA$^+$ gene products, is error prone, and is inducible. UV induced mutagenesis is eliminated by mutations in either the the recA (Miura and Tomizawa, 1968; Kondo, 1969; and Witkin, 1969) or lexA (Witkin, 1969; Mount

et al., 1972) genes of E. coli, which also cause extreme UV sensitivity, lack of prophage induction by UV irradiation (Defais et al., 1971), and allow no Weigle reactivation (Miura and Tomizawa, 1968; Defais et al., 1971). The mutant strains do mutate spontaneously and by agents thought to cause only replication errors (Kondo, 1969; Witkin and Parisi, 1974). It has been proposed that the recA gene product is normally under negative control by the lexA gene product, is inducible, and this inducibility is the molecular basis for activation of SOS functions (Sedgwick et al., 1978). These functions are all $recA^+$ dependent, seem inducible, and require de novo protein synthesis. They are called SOS functions because they arise after treatment with DNA damaging agents (Radman, 1974; Witkin, 1976). SOS functions include lysogenic induction of lambda, filamentous growth, bacterial mutagenesis in excision defective mutant strains, and Weigle reactivation; the extra capacity to promote survival and mutagenesis of irradiated infected phages.

It was first proposed that the recA gene product is the "protein X" of 40,000 molecular weight induced in large quantities following treatments which elicit SOS functions since the control of synthesis of both recA and "protein X" are affected in exactly the same manner (Inouye and Pardee, 1970; Sedgwick, 1975, Gudas and Pardee, 1975; McKentee et al., 1976; Gudas, 1976). Since both recA gene product and protein X comigrate in SDS-polyacrylamide gels, it

was concluded that protein X is the product of the bacterial *recA*$^+$ gene. Further work with two dimensional O'Farrell gels and a lambda vector carrying the *recA*$^+$ gene has also shown this to be the case (Emmerson and West, 1977; McEntee *et al.*, 1976). The *recA*$^+$ gene product acts with the *lexA*$^+$ gene product as a corepressor for itself (Gudas and Pardee, 1975), cleaves the lambda repressor (Shinagawa and Itoh, 1973; Roberts and Roberts, 1975), and stabilizes the structure of DNA (Tomizawa and Ogawa, 1968), especially in the presence of stalled replication forks and DNA repair sites by binding to single stranded DNA (Gudas and Pardee, 1976).

More recent work (Weinstock *et al.*, 1979) has shown that a nearly homogeneous preparation of the *recA*$^+$ protein catalyses an ATP-dependent renaturation of single strands of DNA and, therefore, the protein is probably a "windase" or "annealase" which could play a role in both recombination and DNA repair. It has been shown that the protein allows single stranded DNA to invade homologous super helical DNA, forming D loops, a known substrate for some endonucleases, such as the *recBC* endonuclease (Bridges, 1979). The activities of the *recA* protein are complex, combining proteolytic activity, DNA-dependent ATPase activity, and ATP-dependent "annealase" activity. Furthermore, it seems to control its own synthesis and induction as well.

B. DNA repair in yeast.

Much of the initial work in eucaryotic DNA repair mechanisms began using the procaryotic DNA repair mechanisms such as those characterized in E. coli as models. The simple eucaryotic baker's yeast, Saccharomyces cerevisiae, like E. coli, is known to have many genetic loci involved in DNA repair after treatment with DNA damaging agents such as UV light, ionizing radiation and chemical mutagens (Averbeck et al., 1970; Cox and Parry, 1968; Game and Mortimer, 1974; Lemontt, 1971; Nakai and Matsumoto, 1967; Prakash, 1977a, 1977b; Resnick, 1969; Snow, 1967). Yeast cells can photoreactivate UV induced pyrimidine dimers, and a mutant unable to carry out photoreactivation has been isolated (Resnick, 1969). Another pathway is responsible for excision of pyrimidine dimers in nuclear DNA, and was first indicated by results which show that the ability to photoreactivate a fraction of cell killing is lost over a period of twenty-four hours after irradiation (Parry and Cox, 1968; Parry and Parry, 1969; Patrick, Haynes and Uretz, 1964). The rad1 (Prakash, 1975; Unrau, Wheatcroft, and Cox, 1971; Waters and Moustacchi, 1974), rad2 (Resnick and Setlow, 1972) rad3, rad4 (Prakash, 1977a), rad10, and the rad16 (Prakash, 1977b) loci seem to be involved in the excision repair of UV induced pyrimidine dimers. Excision repair acts only on nuclear DNA and does not remove dimers from mitochondrial DNA (Prakash, 1975; Waters and Moustacchi, 1974). Mutant strains defective in excision repair do

not exhibit sensitivity to X-rays nor do they have any effect on recombination, meiosis or sporulation. Mutants of rad1, rad2, rad3, and rad4 exhibit enhanced UV reversion of a variety of loci tested (Averbeck et al., 1970; Lawrence and Christensen, 1976; Moustacchi, 1969; Resnick, 1969; Zakharov, Kozina, and Fedorova, 1970), a phenotype of excision defective strains. The induced mutability is photoreversible (Averbeck et al., 1970; Lawrence and Christensen, 1976; Resnick, 1969) and, therefore, a result of pyrimidine dimers.

In addition to photoreactivation and excision repair, yeast appears to possess a complex cluster of activities which influence mutagenic repair of UV induced damage involving the function of many genes. Included among these are rev1, rev2, rev3 (Lemontt, 1971), rad6, rad9, rad18 (Lawrence et al., 1974; Lawrence and Christensen, 1976). As in E. coli, UV induced mutagenesis in the eucaryotic yeast, Saccharomyces cerevisiae may be an enzymatic process, with mutations arising as the result of errors made during the repair of damaged DNA. The evidence for the occurrence of mutagenic repair in yeast is the discovery of the above mutations whose phenotypes include increased sensitivity to the lethal effects of UV and X-ray irradiation and a marked reduction in UV induced mutability compared to wild type at all test loci examined (Lawrence et al., 1974, Lawrence and Christensen, 1976; Lawrence and Christensen, 1978a, 1978b; Lemontt, 1971). Lemontt (1973) has

isolated seven additional mutations, designated umr, which decrease UV mutagenesis. Mutant strains of three of these, umr1, umr2 and umr3, are slightly more UV sensitive than wild type strains and affect the UV revertability of one or more of the three alleles examined (Lemontt, 1977). One or more of these may be involved with specific branches of mutagenic repair pathways although their effect on the overall repair capacity of the cell is small.

The RAD6 gene activity (the importance of the RAD6 gene will be discussed at length in a later section) appears to be necessary for all kinds of mutagenic events at all sites within the genome, while the products of other genes that act in this mutagenic cluster of activities have a more restricted role and are involved in only some kinds of mutations. An example of genes which have a more restricted role are the REV1 and REV2 gene products. The REV1 gene function is not required for the production of most base pair additions or deletions and although necessary for the formation of most base pair substitutions is not required for the substitutions which lead to the reversion of cyc1-131, and the proline missense mutation cyc1-115 (Lawrence and Christensen, 1978a, 1978b). The REV2 gene product seems to be involved only with the UV reversion of ochre alleles (Lawrence and Christensen, 1978b).

Eight mutant loci, rad50 to rad57, have been identified which principally affect X-ray sensitivity in yeast (Game and Mortimer, 1974). Gamma ray induced reversion frequencies in these mutant

strains are similar to those of wild type strains indicating an error free mechanism of DNA repair (McKee and Lawrence, 1979). Some of the genes involved in X-ray resistance are also involved in cellular processes such as meiosis (Cox and Parry, 1968; Nakai and Matsumoto, 1967; Resnick, 1969). The best studied mutant of the eight is rad52, which seems to be required for the rejoining of DNA double strand breaks after X-ray induced chromosome breaks (Resnick, 1969), and may involve recombinational repair.

Other mutants are though to be involved in DNA repair, such as MMS sensitive mutants (Prakash and Prakash, 1977) and cell cycle mutants, cdc8 and cdc9 (Prakash et al., 1979; Johnston, 1979). The DNA replication mutant of yeast, cdc8, has been shown to decrease UV induced mutagenesis although in vitro experiments have shown it is not defective for either DNA polymerase I or DNA polymerase II (Prakash et al., 1979). The cdc9 cell cycle mutant which is defective in DNA ligase also is defective in the repair of DNA damaged by UV irradiation (Johnston and Nasmyth, 1978), and is sensitive to MMS but relatively insensitive to gamma irradiation (Johnston, 1979).

C. The RAD6 gene

As mentioned earlier, the RAD6 gene of Saccharomyces cerevisiae appears to be essential for a variety of processes that enhance survival and mutagenesis in DNA damaged cells. The

characteristics, regulation and number of these processes is not yet fully understood. Mutant strains carrying the rad6-1 allele are more sensitive than wild type strains to a variety of physical and chemical agents (Cox and Parry, 1968; Lawrence et al., 1974), are deficient in induced mutagenesis after treatment with these agents (Hunnable and Cox, 1971; Kern and Zimmermann, 1978), exhibit elevated levels of spontaneous and induced recombination (Hastings et al., 1976), and are sensitive to growth inhibition by trimethoprim, an antifolate drug (Game et al., 1975). Diploids homozygous for rad6-1 and rad6-3 do not sporulate (Cox and Parry, 1968; Game and Mortimer, 1974; Douthwright-Fasse, unpublished data, see Appendix II), and rad6-1 mutant strains do not allow meiotic recombination although normal premeiotic DNA synthesis is carried out (Game, personal communication). Haploid strains carrying rad6-1 are unable to repair single and double strand breaks induced in the DNA by MMS (Chlebowicz and Jachymczyk, 1979; Jachymczyk et al., 1977). Since the repair of double strand breaks in wild type haploid strains occurs only in G_2 it seems likely that this occurs by a recombination dependent mechanism (Jachymczyk et al., 1977).

The RAD6 gene is one of at least nine, and perhaps as many as fourteen genes that form a cluster of activities involved in mutagenic DNA repair (Lawrence and Christensen, 1976). Other strains within the epistasis group never exhibit all of the above

phenotypes and any one of the phenotypes is rarely as extreme as in rad6 mutant strains (Cox and Parry, 1968; Lawrence and Christensen, 1976; Lawrence, and Christensen, 1978a). This information suggests that there may be a number of independent and diverse processes that are dependent on the RAD6 gene product. It is possible that the product of the RAD6 gene may act in a regulatory manner, rather than enzymatically, in order to control such diverse processes. Since both the rad6-1 and rad6-3 alleles can be translationally suppressed, it seems likely that the gene codes for a protein product (Lawrence and Christensen, unpublished data).

That the RAD6 gene product is active in a variety of different processes has been investigated in several ways. Attempts to separate these functionally distinct groups have been made in three ways, first by examining the phenotypes of strains that carry one or more of the mutations in the cluster (Lawrence et al., 1974; Lawrence and Christensen, 1976; 1978a, 1978b); second by the isolation of metabolic supressors of rad6 mutations (Lawrence and Christensen, J. Bact., in press) and finally by characterizing a new mutation of the allele, rad6-4 as described in this thesis.

D. Photoreversibility

Photoreversibility has been used by other workers and in this study as a probe to investigate the initial events leading to recovery from lethal damage or mutagenesis. The probe is useful

since the photoreactivating enzyme acts with high specificity to split cyclobutyl pyrimidine dimers (Setlow, 1966), and because of the ease with which photoreactivation can be applied. Witkin (1966a, 1966b) has suggested that relatively few mutations result from errors in repair after normal excision of pyrimidine dimers. She proposes that most mutations arise from replication errors due to the presence of unrepaired damage in the DNA, because the frequency of UV induced try$^+$, lac$^-$, and STR-R mutations at a given dose is greatly elevated in strains of E. coli lacking the capacity for dimer excision compared to strains that are excision proficient. Nishioka and Doudney (1969) continued work in this area to determine the role of error in the excision repair process in causing mutations in wild type and radiation resistant E. coli. Their studies examined the loss of photoreversiblity of UV induced mutation to STR-R and tryptophan independence with hcr$^-$ E. coli lacking excision repair. They demonstrated that although loss of photoreversibility to STR-R occurs in hcr$^+$ strains, in hcr$^-$ strains LOP is concurrent with semiconservative DNA synthesis. Similar results were found when examining the effect of photoreversibility on survival after UV irradiation. These results indicate that excision repair is responsible for the loss of photoreversibility in hcr$^+$ strains, but in hcr$^-$ strains the loss of photoreversibility occurs through a process requiring DNA replication.

We have utilized photoreversibility in a similar manner for this study in the yeast system, and our data indicate that different physiological conditions are required for LOP in yeast than in *E. coli*. *De novo* protein synthesis is required for LOP in excision defective yeast strains, but not DNA synthesis. Therefore yeast differ from *E. coli* in the mechanism available to deal with repair during the first cell cycle following DNA damage.

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CHAPTER ONE

A NEW ALLELE OF THE *RAD6* GENE IN YEAST THAT ALLOWS SPORULATION AND REVERSION

by

Jean Douthwright-Fasse, Roshan B. Christensen and

Christopher W. Lawrence

(Submitted to: *Genetics*)

ABSTRACT

Strains carrying a new allele, designated rad6-4, of the RAD6 locus of Saccharomyces cerevisiae are about as sensitive to UV and ionizing radiation as those carrying rad6-1 or rad6-3 but, unlike them, are capable of induced mutagenesis and sporulation. Diploids homozygous for rad6-4 or heteroallelic for rad6-4 and either rad6-1 or rad6-3 allow UV induced reversion of the ochre alleles arg4-17 and cyc1-9, of the proline missense allele cyc1-115, and of the frameshift allele cyc1-239. They also permit EMS induced reversion of cyc1-115. The frequency of reversion is greater than wild-type in each case. Homozygous rad6-4 diploids sporulate as well as wild type, while heteroallelic rad6 diploids sporulate less well than wild-type. Although rad6-4 may well be a missense mutation, our evidence shows that it is unlikely that this phenotype is due to leakiness. Instead, the data suggest that the RAD6 gene is multifunctional. One function is necessary to recover from DNA damage in an error-free manner, and the other is concerned with mutagenic processes and sporulation. Rad6-1 and rad6-3 strains are deficient in both of these functions, while rad6-4 strains are deficient only in the error-free function.

INTRODUCTION

The RAD6 gene is concerned with a variety of important functions necessary for survival and mutagenesis in Saccharomyces cerevisiae. This organism has a variety of repair processes in addition to photoreactivation of pyrimidine dimers to deal with radiation damage (BRENDEL and HAYNES 1973; COX and GAME 1974); one that leads to the excision of damage from DNA (UNRAU, WHEATCROFT and COX 1971; RESNICK and SETLOW 1972; PRAKASH 1977), a second thought to promote repair of double strand breaks by a recombination dependent mechanism (RESNICK and MARTIN 1976), a third concerned with mutagenesis and recovery from damage caused by a variety of agents that is dependent on mechanisms as yet not well understood (LAWRENCE and CHRISTENSEN 1974; PRAKASH 1974; LEMONTT 1971).

The RAD6 gene appears to be one of the most important among the group involved in carrying out the third of these processes. Strains carrying mutant alleles of the RAD6 gene exhibit a wide variety of phenotypes; sensitivity to many DNA damaging agents such as radiation and chemicals, lack of mutagenesis induced by such agents, sensitivity to the folate antagonist trimethoprim, and lack of sporulation in homozygous diploids. Strains carrying mutations in other genes that affect this process never exhibit all of these phenotypes, and any one mutant phenotype is rarely as extreme. These properties suggest that the RAD6 gene function is important for a variety of processes, and raises the possibility that the

gene is multifunctional. This possibility has been investigated in two ways. First the isolation and study of metabolic suppressors of the rad6-1 mutation (LAWRENCE and CHRISTENSEN, in preparation), and second, an examination of the phenotype of a new RAD6 mutant allele, designated rad6-4.

Strains carrying rad6-4, are about as sensitive as those carrying rad6-1 or rad6-3, but unlike these strains are capable of induced mutagenesis and sporulation. Such results indicate that the RAD6 gene function is involved in at least two separate and independent activities. One activity, or set of activities, is recovery from DNA damage in a non-mutagenic manner. This activity is lacking in strains carrying any one of the three rad6 alleles and accounts for the extreme sensitivity to UV and ionizing radiation of these mutants. The second group of activities are those responsible for induced mutagenesis and sporulation. The rad6-4 strains seem to possess all of the mutagenic processes found in a wild type strain, while rad6-1 strains possess none of them.

MATERIALS AND METHODS

Mutant Isolation: The procedure to isolate the rad6-4 mutant was that of PRAKASH and PRAKASH (1977). The parental strain B-635 (a cyc1-115 his1 lys2 trp2) was mutagenized by 130 Jm^{-2} UV irradiation giving 3% survival. 4824 surviving clones were picked onto master plates and tested. Each master plate was tested for sensitivity to MMS at 23° and 35°. 400 presumptive mutants that appeared to be MMS sensitive at one or both temperatures, were examined further for sensitivity to UV irradiation, ionizing radiation and MMS, at 23°C and 35°C. They were also screened for UV-induced reversion of lys2-1 and sensitivity to trimethoprim. One unconditional rad6 mutant was found among the 400 clones.

To assay for ochre or amber suppression in rad6-4 strains, haploids containing the radiation sensitive allele and ochre or amber suppressible auxotrophic markers were crossed to strains carrying SUP7-1, an ochre suppressor, or SUP7-2, an amber suppressor. The diploids were then sporulated, dissected, and tested for suppression of the ochre or amber markers and the rad6 phenotype of radiation sensitivity.

Strains: Three series of diploid strains were constructed to investigate the properties of the new rad6-4 and the previously isolated rad6-1 (COX and PARRY 1968) and rad6-3 (PRAKASH and PRAKASH 1977) alleles. For the first series, strains carrying one of the three rad6 alleles were backcrossed twice to each of two

parental strains. The parental strains were chosen for their background of good sporulation when intercrossed. The strains were all homozygous for the highly revertible ochre allele arg4-17 (LEMONTT, 1971) to serve as a test allele for UV induced mutagenesis. A second series of strains homozygous for the cyc1-9, cyc1-115, and cyc1-239 alleles of the structural gene for iso-1-cytochrome c were also constructed in order to study UV-induced mutagenesis in rad6-4 homozygous diploids and heterozygous controls. In the final series of strains, rad6-4/rad6-1 heteroallelic diploids were constructed, that were heteroallelic for one of the three cyc1 point mutations and cyc1-1, a non-reversible deletion of the whole CYC1 locus (PARKER and SHERMAN 1969; SHERMAN et al. 1975). In most cases the diploids were isolated by prototrophic selection, and their diploid status verified by sporulation. This test was not possible in the cases of homoallelic rad6-1 or rad6-3 strains, and heteroallelic rad6-1/rad6-3 strains. Replicate strains were constructed in all cases.

Determination of Reversion Frequency and Surviving Fraction: All strains were grown for three days into late stationary phase, in 10 mls of liquid YPD medium (Difco yeast extract, 1%; Bacto-peptone, 2%; dextrose, 2%) with vigorous shaking at 30°. Cultures were briefly sonicated when necessary to disrupt cell aggregates. Arginine and lysine revertants are detected by spreading up to 1 x 10^7 cells on plates containing synthetic (SD) medium (Difco yeast

nitrogen base without amino acids or ammonium sulfate, 0.6%; ammonium sulfate, 1.8%; dextrose, 7.2%; Difco Agar Noble, 1.5%) and supplemented with the necessary nutrilites. Colonies arising from revertants were scored after 4 - 7 days incubation at 30°. Viability was assayed on SD media containing all requirements including arginine and lysine. Cytochrome c revertants were detected by using semi-synthetic lactate (SLY) medium (Difco yeast nitrogen base without amino acids or ammonium sulfate, 0.17%; ammonium sulfate, 0.5%; Difco yeast extract, 0.05%; DL-lactate, 1%; Difco Agar Noble, 1.5%), and supplemented with the necessary nutrilites. YPG medium, similar to YPD, but containing 3% (V/V) glycerol rather than dextrose and solidified with 1% Difco bacto agar, was used to measure viability for cytochrome c reversion. Samples of cyc1 revertants were examined spectroscopically by the method of SHERMAN and SLONIMSKI (1964) to confirm that the scoring of intragenic revertants was accurate. The plating densities used were generally low enough to avoid density-dependent artifacts (LAWRENCE and CHRISTENSEN, 1976).

Reversion frequency was estimated at doses of 3.5 and 7.0 Jm^{-2}, fluences chosen to give at least one comparison between wild-type and radiation sensitive strains at a dose giving relatively high survival. Details regarding the UV radiation source and its dosimetry are given in LAWRENCE et al. (1974). Gamma-ray exposure was carried out in a ^{60}Co irradiator (J.L. Shepherd and Associates) in aerated liquid cultures before plating. Dosimetry measurements and irradiation methods are given in MCKEE and LAWRENCE (in press).

Trimethoprim resistance: Trimethoprim resistance was assayed by plating between 10^2 and 10^3 cels on SD medium containing trimethoprim (2,4-diamino-5[3,4,5-trimethoxy benzl]pyrimidine, 200 µg/ml) and also on unsupplemented SD control plates.

Sporulation: Sporulation frequency was examined twice in the first series of diploids (see under "Strains") by growing them on solid YPD medium for three days and transfering them to slants containing sporulation medium (Potassium acetate, 2%; dextrose, 0.1%; Difco yeast extract, 0.25%; Difco Agar Noble, 1.67%) supplemented with a nutrilite solution. The number of asci in a total of from 500 to 2000 cells was counted in each strain after incubation for seven days at 30°.

RESULTS

Isolation and Identification: 400 mutant clones judged to be MMS sensitive at either 23° or 35° were screened for their ability to complement rad6-1, rad18-2, and rad52-1, for UV induced reversion of lys2-1 and for sensitivity to trimethoprim. A mutation in one of these isolates, A-270, did not complement either rad6-1 or rad6-3 for sensitivity to UV or ionizing radiation (see Figures 3 and 6), and the strain exhibited growth that was sensitive to inhibition by trimethoprim, a property found only in rad6 and rad18 mutants (GAME et al. 1975). Crosses of A-270 to wild-type strains indicated that the UV sensitivity of this strain was due to a single recessive gene. Finally, forty tetrads from a rad6-1, rad6-4 heteroallelic diploid yielded 4:0 segregation for the UV sensitivity phenotype indicating that the mutation in A-270 was located less than 1cM from the rad6-1 site. We conclude that the mutation in A-270 is therefore an allele of the RAD6 gene, which we designate rad6-4.

Radiation Sensitivity: Haploid and diploid strains carrying the rad6-4 allele are about as sensitive to both UV and ionizing radiation as comparable strains carrying the rad6-1 and rad6-3 alleles. Figures 1-6 show the average survival curves of haploid strains, and also of homoallelic and heteroallelic diploid strains, with similar genetic backgrounds, after treatment with UV and ionizing radiation. All of the rad6 strains are very sensitive to irradiation compared to wild type controls. Although rad6-4

FIGURE 1 Average UV survival curves for RAD+ (JF207-1B,; JF147-1C), rad6-1 (JF207 -10C, JF147-2B), rad6-3 (JF86-6C, JF152-1C) and rad6-4 (JF96-26B, JF185-1D) haploid strains.

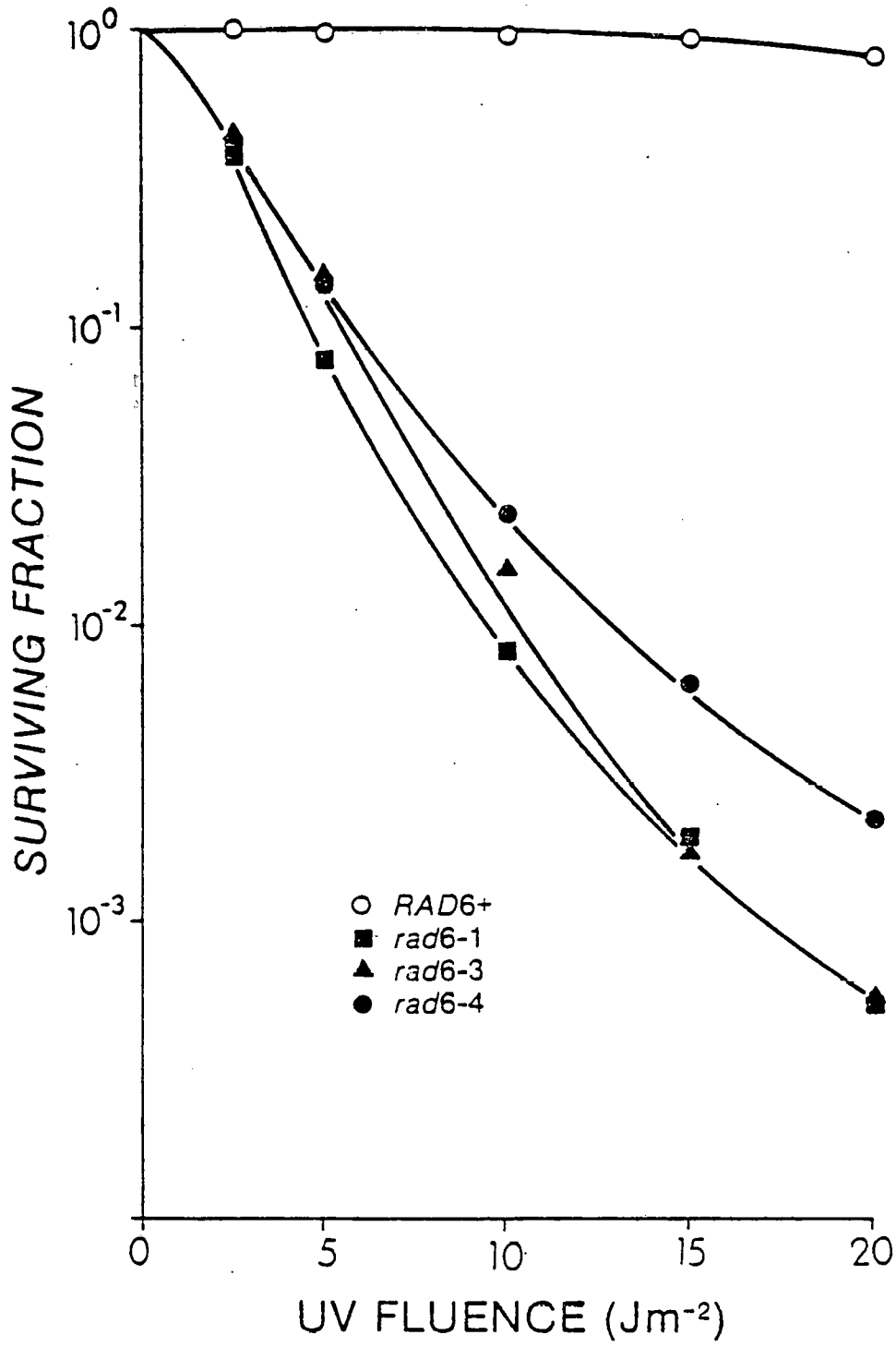

SURVIVING FRACTION
10^0
10^{-1}
10^{-2}
10^{-3}
RAD6+
rad6-1
rad6-3
rad6-4
0
5
10
15
20
UV FLUENCE (Jm^{-2})

FIGURE 2 Average UV survival curves for RAD+/rad6-x (r172, r23), rad6-1/rad6-1 (r20, r95), rad6-3/rad6-3 (r153, r173) rad6-4/rad6-4 (r322, r320) diploids.

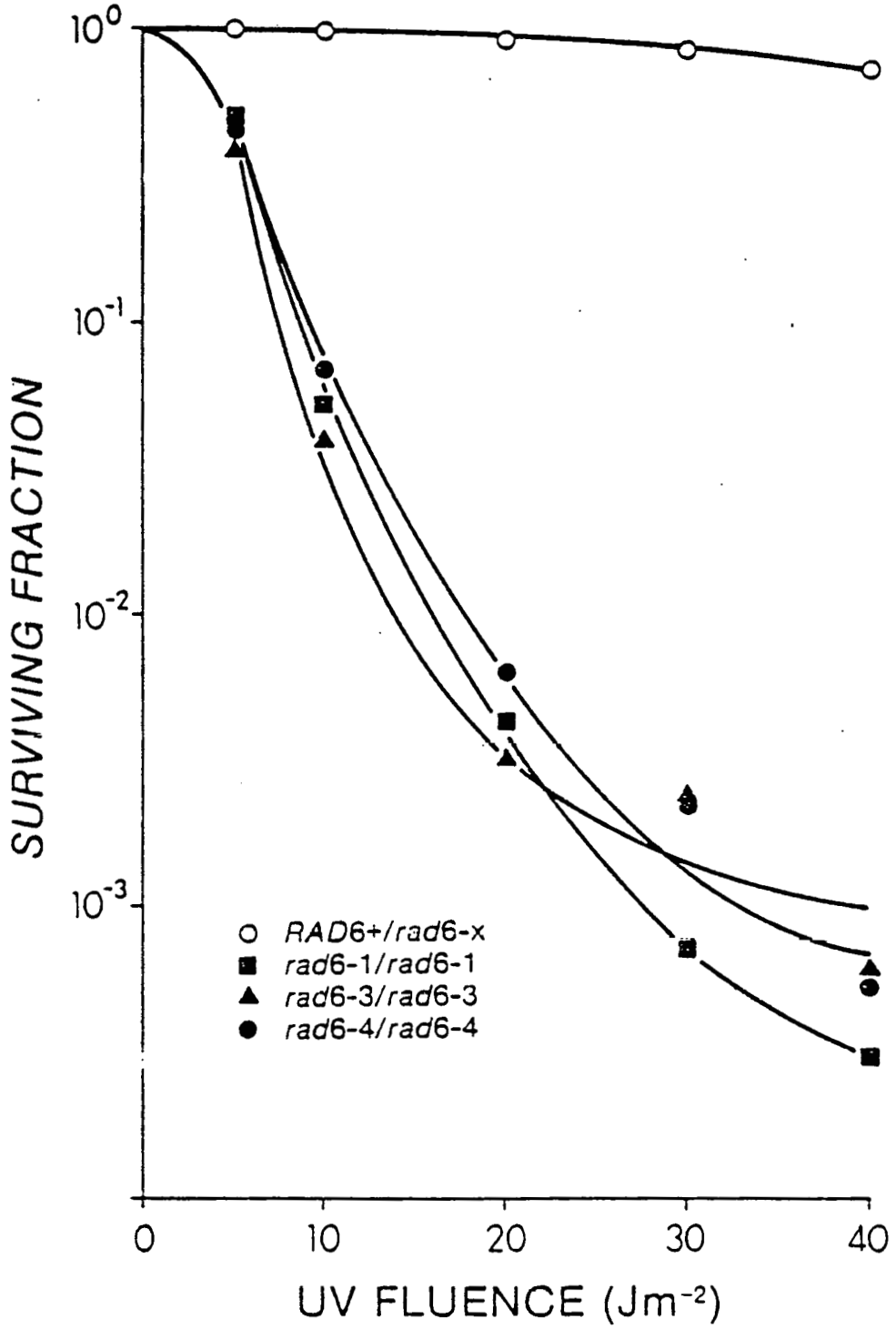

SURVIVING FRACTION
10^0
10^{-1}
10^{-2}
10^{-3}
RAD6+/rad6-x
rad6-1/rad6-1
rad6-3/rad6-3
rad6-4/rad6-4
0
10
20
30
40
UV FLUENCE (Jm^{-2})

FIGURE 3 Average UV survival curves for RAD+/rad6-x (r172, r23), rad6-3/rad6-4 (r178, r317), rad6-1/rad6-4 (r104, r311) and rad6-1/rad6-3 (r99, r27) heteroallelic diploids.

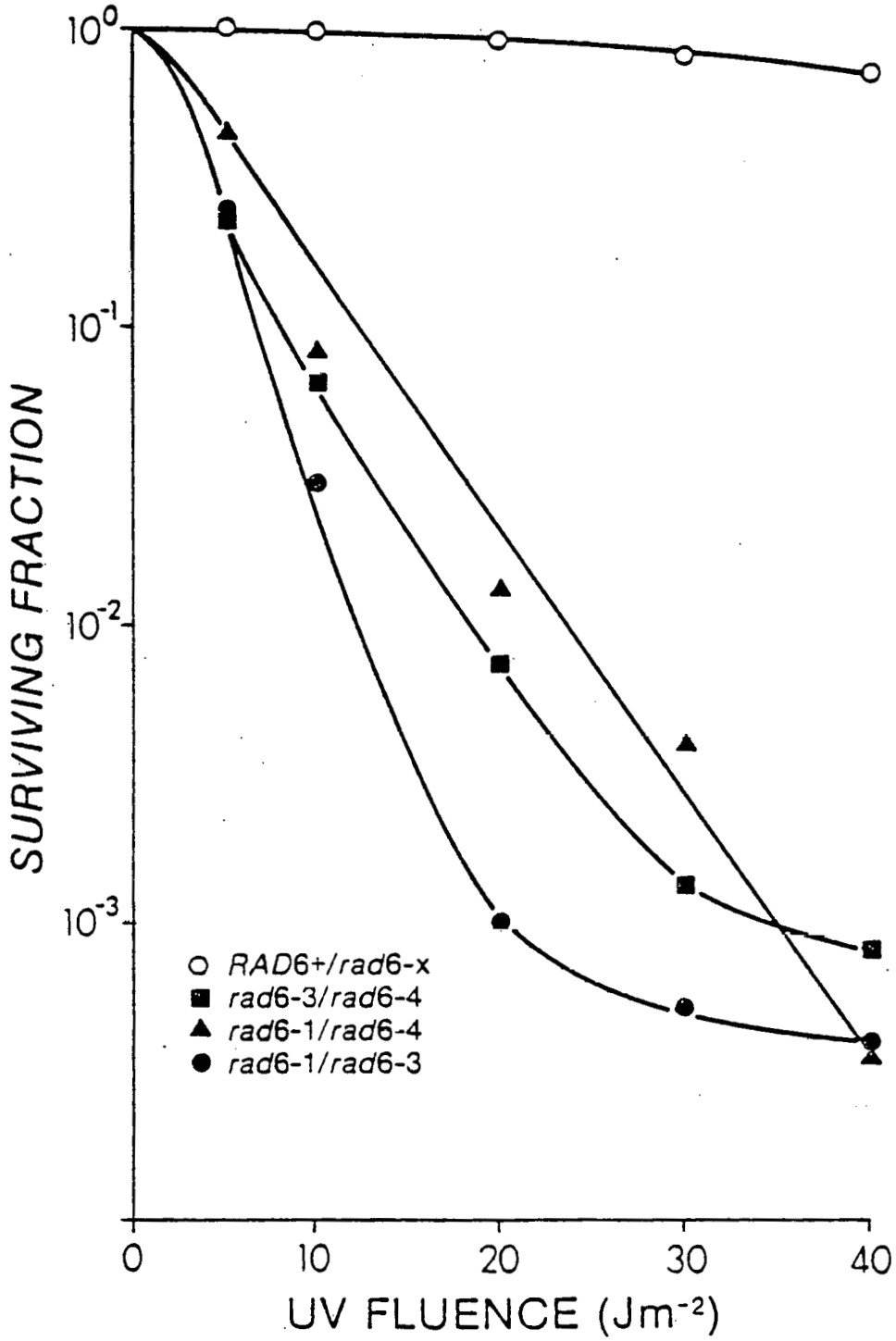

SURVIVING FRACTION
10^0
10^{-1}
10^{-2}
10^{-3}
RAD6+/rad6-x
rad6-3/rad6-4
rad6-1/rad6-4
rad6-1/rad6-3
0
10
20
30
40
UV FLUENCE (Jm^{-2})

FIGURE 4 Average gamma ray survival curves for RAD+ (JF207-1B, JF147-1C) rad6-1 (JF147-9D, JF207-10C), rad6-3 (JF152-1C, JF86-6C) and rad6-4 (JF96-26B, JF195-4C) haploid strains.

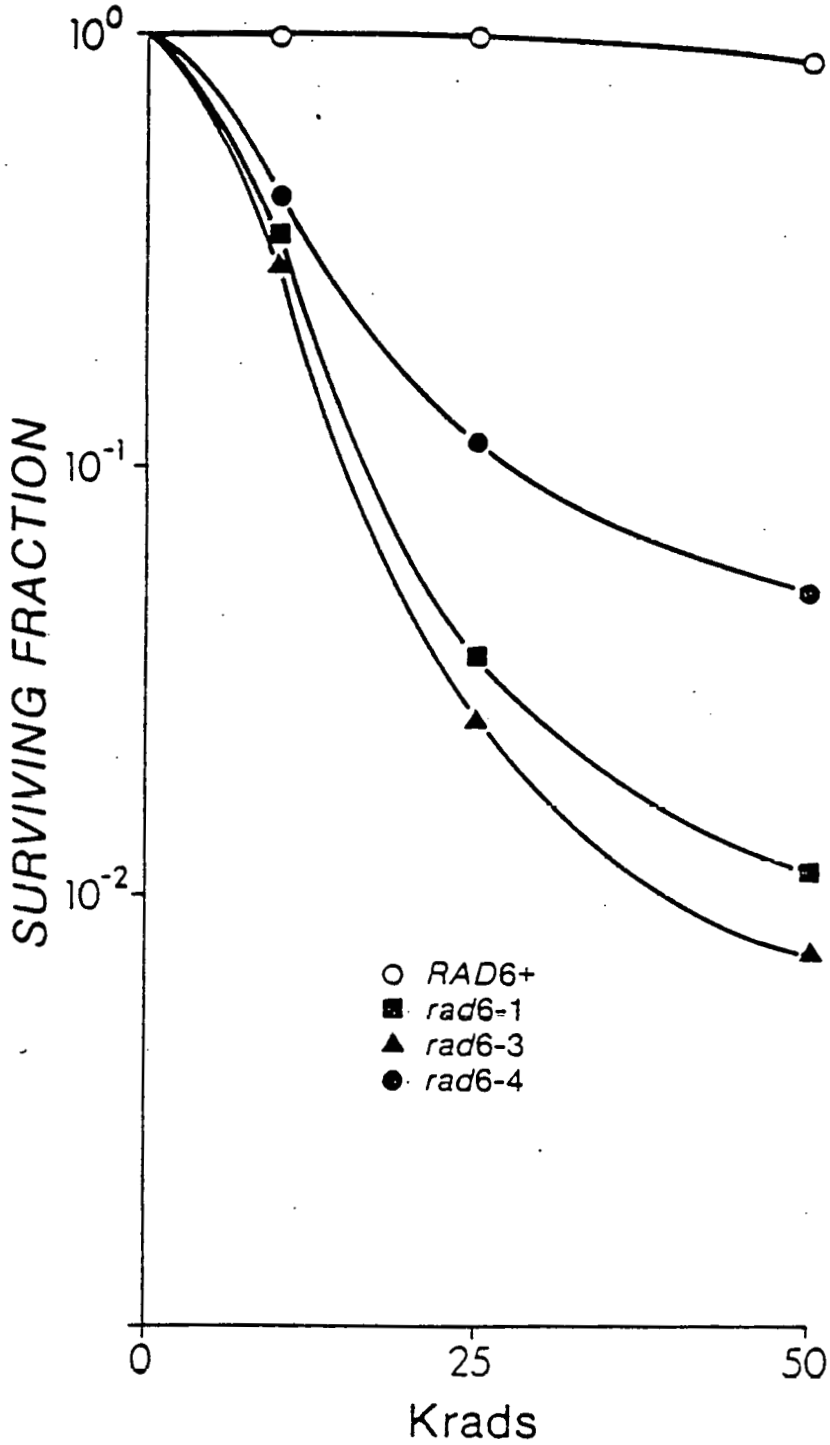

10^0
10^{-1}
10^{-2}
SURVIVING FRACTION
RAD6+
rad6-1
rad6-3
rad6-4
0
25
50
Krads

FIGURE 5 Average gamma ray curves for RAD+/rad-x (r172, r23), rad6-1/rad6-1 (r20, r95), rad6-3/rad6-3 (r173, r153), and rad6-4/rad6-4 (r322, r266) diploids .

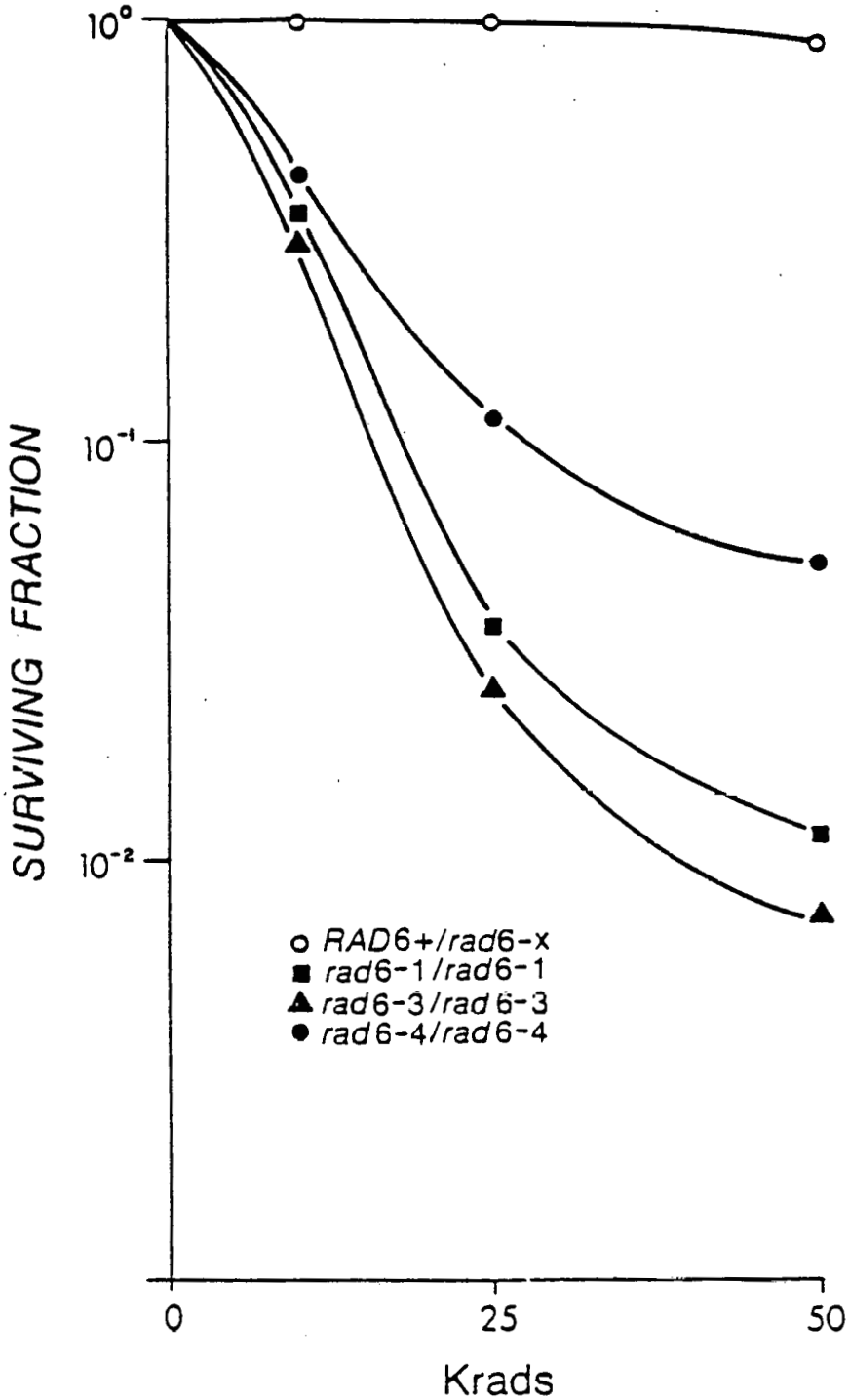
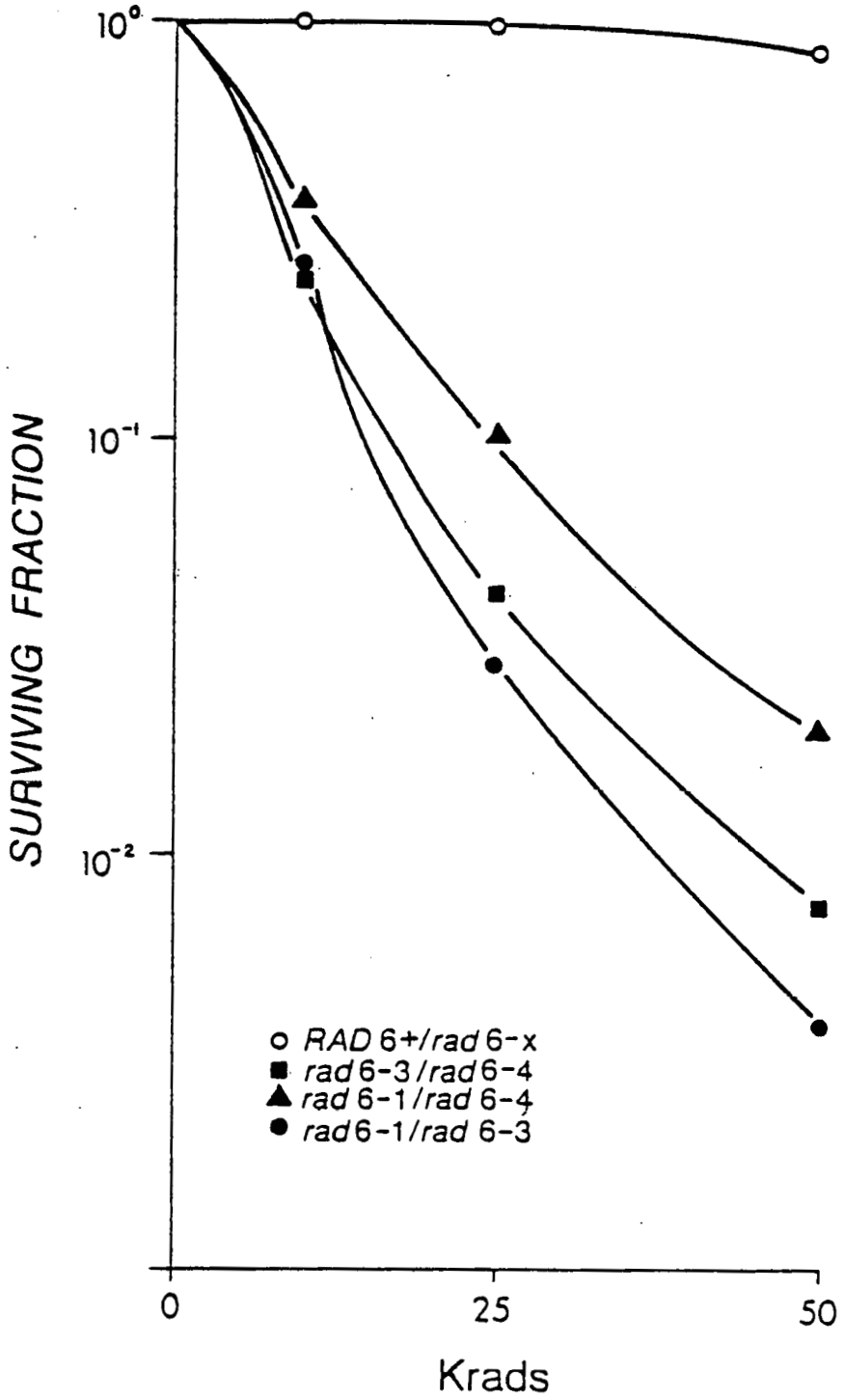

SURVIVING FRACTION
10°
10⁻¹
10⁻²
0
25
50
Krads
RAD6+/rad6-x
rad6-1/rad6-1
rad6-3/rad6-3
rad6-4/rad6-4

FIGURE 6 Average gamma ray survival curves for RAD+/rad6-x (r172, r23), rad6-3/rad6-4 (r317, r178), rad6-1/rad6-4 (r311, r106) and rad6-1/rad6-3 (r167, r146) heteroallelic diploids.


SURVIVING FRACTION
10⁰
10⁻¹
10⁻²
0
25
50
Krads
RAD 6+/rad 6-x
rad 6-3/rad 6-4
rad 6-1/rad 6-4
rad 6-1/rad 6-3

strains may be slightly more resistant to UV radiation than rad6-1 and rad6-3 strains, the difference is at best small and its existence is not supported by an analysis of variance of the log of the surviving fraction. The difference in sensitivity between strains carrying rad6-4 and the other rad6 alleles seems greatest in the gamma ray survival curves. The determination of these sensitivities is made difficult by the presence of a variable sized subpopulation of resistant cells in all rad6 mutant cultures. These subpopulations not only give rise to tails on the survival curves, but also influence the initial slope, and may account for the apparent differences in sensitivities. The resistant subpopulations may in part be the consequence of aggregates of cells that have resisted disruption during sonication, and in part due to the occurrence of various suppressors, which readily accumulate in rad6 mutants due to the growth advantage that they impart to these sick strains.

Induced Mutagenesis: The results given in Tables 1 and 2 not only show that rad6-4 strains are capable of induced mutagenesis, but also suggest that they possess the full range of mutagenic processes found in wild types. Apart from responding mutagenically to two contrasting mutagens, UV and EMS, rad6-4 strains also exhibit UV-induced reversion of four alleles which, as discussed below, revert by partially independent processes.

The four alleles examined were arg4-17, an ochre allele that reverts well with UV (LEMONTT 1971), cyc1-9 an ochre allele of the

TABLE 1

Induced reversion frequency of arg4-17 and cyc1 alleles in diploid strains homozygous or heterozygous for rad6-4 and heteroallelic for rad6-1 and rad6-4 exposed to 3.5 and 7.0 Jm^{-2} UV radiation. Percent survival in parentheses.

	Induced revertants per 10^{-8} survivors (% survival)					
	RAD+/rad6-4		rad6-4/rad6-4		rad6-1/rad6-4*	
allele reverted	3.5 Jm^{-2}	7.0 Jm^{-2}	3.5 Jm^{-2}	7.0 Jm^{-2}	3.5 Jm^{-2}	7.0 Jm^{-2}
arg4-17 (ochre)	31 (103)	94 (101)	309 (73)	1518 (32)	2172 (39)	10931 (7)
cyc1-9 (ochre)	32 (105)	132 (103)	219 (68)	738 (38)	911 (39)	2193 (12)
cyc1-115 (proline missense)	38 (99)	207 (105)	376 (85)	675 (37)	683 (39)	1076 (17)
cyc1-239 (frameshift)	8 (111)	9 (98)	28 (77)	61 (29)	43 (35)	132 (15)

*The homoallelic and heterozygous strains have comparable genetic backgrounds, but the heteroallelic diploids are derived from a different set of crosses.

TABLE 2

Induced reversion frequency of the cyc1-115 allele in diploid strains homozygous or heterozygous for rad6-4 exposed to 0.5% EMS for 0 - 5 hours.

time of treatment (hours)	Induced revertants per 10^{-8} survivors (% survival cyc1-115 → CYC1+)	
	RAD+/rad6-4	rad6-4/rad6-4
1	88 (76)	143 (80)
2	193 (86)	395 (80)
3	418 (89)	794 (78)
4	768 (91)	1587 (71)
5	1156 (82)	2442 (71)

structural gene for iso-1-cytochrome c, cyc1-115, a proline missense mutant; and cyc1-239, a frameshift mutation (SHERMAN et al. 1974). The three cyc1 alleles revert as a consequence of different base pair alterations, and furthermore are representative of groups of alleles that revert by processes that are under partially different genetic control (LAWRENCE and CHRISTENSEN, 1978a, b; 1979). Each of these alleles can be reverted by UV in rad6-4 strains (Table 1), suggesting that these diploids possess the full range of wild type capabilities. EMS induced reversion of cyc1-115 in rad6-4 diploids and in wild type controls is shown in Table 2. As can be seen from this table, EMS is also an effective mutagen in rad6-4 strains. In comparison, rad6-1 strains exhibit no UV or EMS mutagenesis at the sites which have been tested (LAWRENCE et al. 1974; PRAKASH 1974). Although they have not been studied as intensively as rad6-1 strains, rad6-3 diploids also appear to be entirely deficient in UV mutagenesis (DOUTHWRIGHT-FASSE, unpublished data).

Although reversion experiments with rad6 mutants are unusually prone to a variety of selection artifacts, these results are nevertheless likely to reflect the induction, and not selection, of revertants. Thus, there was an absolute increase in the number of revertants per milliliter of irradiated culture, uncorrected for survival. Furthermore, revertants were sampled and shown to be just as sensitive as the parental strain. Arg4-17 can revert by ochre suppression, and this class of mutations has been found previously (LAWRENCE, unpublished data) to give selection

artifacts. Experiments with this allele employed glycerol medium. STEWART et al. (1972) have shown that nonsense suppressors grow poorly on non-fermentable medium, so that little opportunity for the selection of spontaneous suppressors could therefore occur. Each of the cyc1 alleles reverts almost exclusively by intragenic mutation since samples of all revertants were examined spectroscopically (SHERMAN and SLONIMSKI 1964) to confirm that the scoring of these intragenic events was accurate.

The results in Tables 1 and 2 suggest that, qualitatively, the rad6-4 strains possess the same capacity to mutate as wild type strains. Quantitatively, however, reversion frequencies are in all cases increased in rad6-4 strains; for arg4-17, UV-induced reversion in rad6-4 diploids is 10 to 15 fold higher than wild-type, for cyc1-9, about 6 fold higher, for cyc1-115, 3 to 10 fold higher and for cyc1-239, 3 to 6 fold higher. Table 1 shows that UV induced mutagenesis also takes place in rad6-1/rad6-4 heteroallelic diploids. High frequencies of reversion of all the alleles are seen in these strains. Although they are more sensitive to UV irradiation than the rad6-4 homozygous diploids, the heteroallelic diploids and rad6-4 homozygous diploids have different genetic backgrounds, so that comparisons cannot be made. Finally, EMS induced reversion of cyc1-115 is about two fold higher in the rad6-4 strains than in the wild type (Table 2).

Sporulation

The sporulation capacity of strains homozygous, heterozygous or heteroallelic for the different rad6 alleles is given in Table 3. Homozygous rad6-4 strains sporulated to much the same extent as the heterozygous and homozygous wild types, which were statistically homogeneous with respect to sporulation frequency. Neither rad6-1 or rad6-3 homozygous diploids allow any detectable sporulation. Rad6-1/rad6-4 and rad6-3/rad6-4 heteroallelic strains also sporulate, showing that rad6-4 is dominant over the other alleles in this respect.

An analysis of variance of percent sporulation transformed to angles gave three conclusions. First, the homozygous wild type and heterozygous wild type controls were a homogeneous class for sporulation. Secondly, the frequency of sporulation in the homozygous rad6-4 strains was not significantly different from that of the two classes of wild type strains. Finally, the sporulation frequency of rad6-4 heteroallelic diploids was significantly lower than that of the heterozygous wild types, suggesting that the sporulation activity of the rad6-4 gene product may be less than that of the wild type allele, or abnormal in some other way.

Effect of low levels of RAD6 gene product on survival, reversion and sporulation: The data suggest that rad6-4 mutants are almost entirely deficient in error free repair but relatively normal for mutagenic processes and sporulation. This phenotype seems to reflect an inherent property of the rad6-4 allele, dependent on the

TABLE 3

Percent sporulation of diploid strains constructed by intercrossing all combinations of rad6 haploids. Four to seven independent strains were sporulated twice, and 500 cells assayed for the ability to sporulate in rad6-1 and rad6-3 homozygous diploids, and 2000 cells in all other cases.

	RAD6	rad6-4	rad6-3	rad6-1
RAD6	64.0±20.2	42.5±12.2	54.1±14.3	50.6±7.9
rad6-4		44.8±4.9	28.4±7.2	36.6±8.6
rad6-3			0	0
rad6-1				0

organization of the RAD6 gene; even though rad6-4 may well be a missense allele, its phenotype does not appear to be a consequence of leakiness. Strains carrying rad6-4 are as sensitive, or almost as sensitive, to radiations as those carrying the nonsense alleles rad6-1 or rad6-3. Moreover, it is unlikely that lower levels of RAD6 gene product are required for normal sporulation and mutagenesis than for normal survival. A homozygous rad6-1 strain heterozygous for an amber suppressor exhibits a large increase in resistance to UV irradiation, and also shows UV-induced reversion of cyc1-9. These results, shown in Table 4, suggest that a small amount of RAD6 gene product proportionally affects both survival after UV irradiation and UV induced mutagenesis. Sporulation was not observed in this strain, probably the consequence of a bad genetic background. Amber suppression in another rad6-1 strain was found to have a proportionately smaller effect on mutation than on survival, while sporulation was normal (LAWRENCE and CHRISTENSEN, in preparation). The observation that amber suppression in rad6-1 strains does not disproportionately enhance sporulation and mutagenesis suggests that the phenotype of the rad6-4 allele is not due to its leakiness.

Trimethoprim Sensitivity: GAME et al. (1975) showed that rad6-1, rad6-2 (since lost) and rad18 strains are sensitive to growth inhibition by the folate antagonist trimethoprim. This sensitivity appears to depend on the deficiency of rad6-1 and rad6-3 mutant strains with respect to the non-mutagenic repair of UV damage, the deficiency which is responsible for their extreme sensitivity to

TABLE 4

Effect of an amber suppressor on UV-induced reversion of cyc1-9 and survival in diploid strains homozygous for rad6-1.

Strain		Induced revertants per 10^{-8} survivors (% survival) cyc1-9 → CYC1+	
		20 Jm^{-2}	40 Jm^{-2}
rad6-1 / rad6-1	sup+ / sup+	0 (1.7)	0 (0.1)
rad6-1 / rad6-1	sup+ / SUP	544 (71)	2060 (25)
rad6-1 / RAD6+	sup+ / SUP	2732 (95)	5577 (94)

this radiation (LAWRENCE and CHRISTENSEN, in preparation). The growth of rad6-1 or rad6-3 strains that carry the dominant, non-translational suppressor mutation SRS2-1 is not inhibited by trimethoprim, and such strains are also much more resistant to UV than unsuppressed rad6 mutants (LAWRENCE and CHRISTENSEN, in preparation). This suggests that rad6-4 mutants should also be sensitive to trimethoprim, and this was found to be the case; the growth of A-270 and also of eight rad6-4 segregants was inhibited by this drug. Similarly, the trimethoprim sensitivity, and also much of the UV sensitivity, of rad6-4 strains is suppressed by the SRS2-1 mutation (data not shown).

DISCUSSION

The difference in phenotypes between rad6-1 or rad6-3 mutants, and strains carrying rad6-4 strains can be best explained by the hypothesis that RAD6 dependent processes are of at least two kinds; the first non-mutagenic and very important for the survial of wild type strains, the second mutagenic and relatively unimportant for survival, though necessary for sporulation.

Like rad6-1 and rad6-3 mutants, rad6-4 strains are extremely sensitive to DNA damaging agents such as UV, ionizing irradiation and EMS, and moreover are sensitive to about the same extent. Unlike rad6-1, and probably rad6-3 mutants, rad6-4 strains exhibit UV and EMS induced reversion of all alleles examined, at frequencies that are in fact greater then in the wild type, and also sporulate. These abilities of rad6-4 strains suggest that the RAD6 gene may possess several activities. One activity is necessary to recover from DNA damage in a non-mutagenic manner. Strains carrying any of the three rad6 alleles must be deficient in this type of DNA repair due to their extreme sensitivity to DNA damaging agents. The other activity is required for a mutagenic process, or set of processes, and also sporulation. Only rad6 strains carrying the rad6-4 allele exhibit mutagenic repair. Similarly, sporulation of rad6 strains is possible only when the rad6-4 allele is present.

Strains carrying rad6-4 possess several, and probably all, of the mutagenic capabilities of wild type strains. There is evidence for several branches of RAD6 dependent mutagenic repair in yeast, some site specific, and others specific for certain base pair changes or for mutagenesis induced by particular mutagens (LAWRENCE and CHRISTENSEN, 1978a, b; 1979; MCKEE and LAWRENCE, in preparation). The three cyc1 alleles which were utilized to examine UV induced mutagenesis, cyc1-9, cyc1-115, and cyc1-239 are representative of alleles that revert by partially independent mutational processes. UV and EMS were utilized as agents to assay for mutagenesis induced by contrasting types of mutagens (PRAKASH 1976). All strains containing rad6-4 allowed induced mutagenesis by these contrasting mutagens, as judged by the reversion of the alleles tested. Induced reversion frequencies were in fact in all cases higher in rad6-4 strains than in wild types, in a manner reminiscent of UV-induced mutation in excision defective strains. The reason for this is not known, but may also depend on the diversion of a greater amount of damage into mutagenic recovery processes due to the absence of non-mutagenic RAD6 repair mechanisms in rad6-4 strains.

The rad6-4 allele is dominant over either the rad6-1 or rad6-3 alleles for sporulation. The homozygous rad6-4 diploid strains sporulate, although neither the rad6-1 or rad6-3 homozygous diploids exhibit any detectable sporulation ability. Diploids heteroallelic for rad6-4 and either rad6-1 or rad6-3 also

sporulate, but with significantly lower frequency than the heterozygous wild types. These data suggests that the sporulation activity of the rad6-4 gene product may be less than that of the wild type allele, or altered in some way. GAME et al. (personal communication) have shown that rad6-1 strains are deficient in recombination in the period between premeiotic DNA synthesis and commitment to sporulation. This could mean that the RAD6 gene product is required for recombination itself, or that it is required for some other essential step in sporulation that precedes recombination. Rad6-4 strains are presumably not deficient in recombination in this period since they sporulate, and give viable ascospores.

Apart from being a major pathway for repair of damage from UV and ionizing radiation, non-mutagenic repair is also responsible for trimethoprim resistance in wild type strains. The lack of non-mutagenic repair confers sensitivity to the drug in strains carrying all three rad6 alleles. The SRS2-1 mutation, which suppresses the UV and trimethoprim sensitivity of rad6-1 and rad6-3 strains, also suppresses these properties in rad6-4 strains.

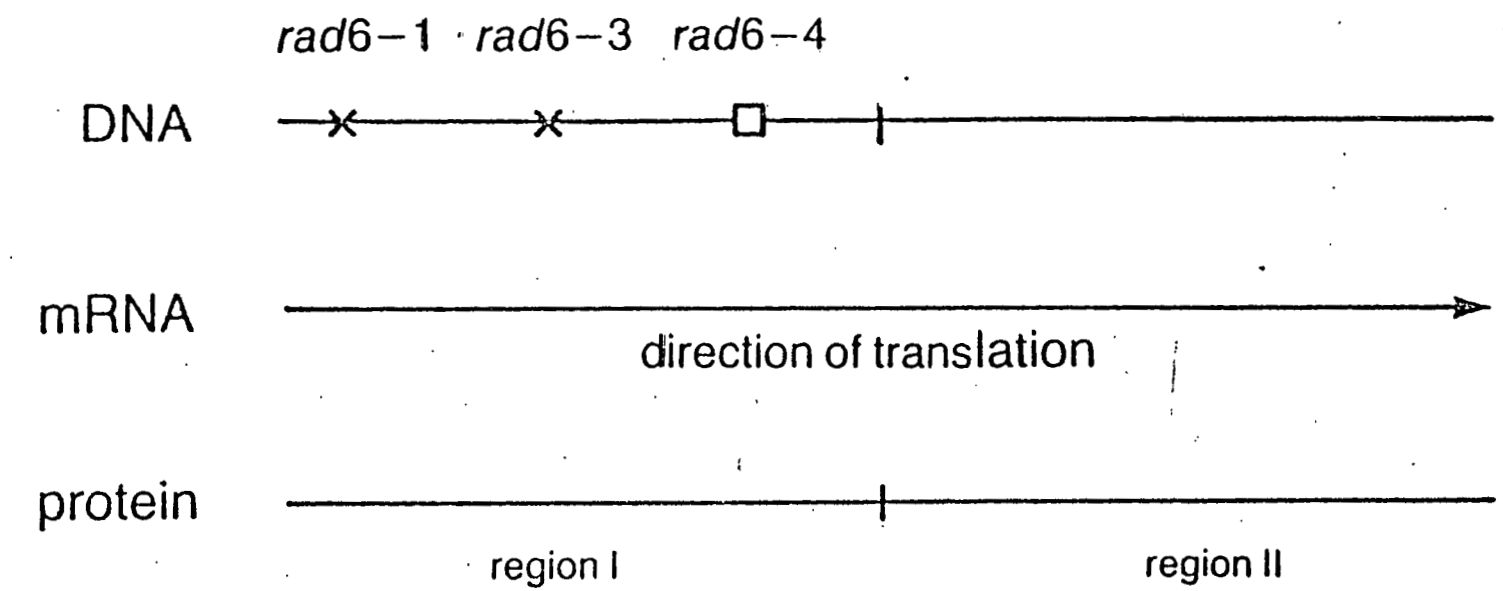
It is unlikely that the results obtained from strains carrying the rad6-4 allele are due to "leakiness" of this allele even though it is probably a missense allele, in contrast to the other two alleles, which are nonsense mutations. First, rad6-4 strains are as sensitive, or at least almost as sensitive as strains carrying rad6-1 or rad6-3. Moreover translational suppression of the rad6-1

allele does not enhance the mutagenic or sporulation capacity of the strain to an any greater extent than its UV resistance. Although some amber suppressors have an efficiency as high as 75% (LIEBMAN and SHERMAN 1976), most suppress with an efficiency of only about 15% at most. The efficiency with which rad6-1 was suppressed in the present strain is not known, but it is unlikely to be high since the strain did not grow as poorly as those in which suppression is very efficient. A small amount of RAD6 gene product would therefore appear to affect both induced mutagenesis and UV sensitivity hand in hand. It is not known, however, whether the amino acid inserted by the amber suppressors are equally effective at restoring both of these activities in the RAD6 product.

In conclusion, the data suggest that the rad6 gene is multifunctional. Three alleles exist, and all are deficient in non-mutagenic repair of DNA damage. One allele, rad6-4, allows mutagenic recovery from DNA damage, and sporulation. This correlation suggests that similar, perhaps identical activities are concerned with mutagenesis and some step in the process of sporulation that could be recombination. Figure 7 shows a possible model of the RAD6 gene. It is suggested that the gene codes for two activities, or at least that different parts of the RAD6 polypeptide play varying roles in two kinds of activities. The rad6-1 and rad6-3 alleles are nonsense mutations and no functional protein is translated. The rad6-4 allele allows a protein to be translated, but it contains a missense mutation which abolishes one

FIGURE 7 Proposed model of the RAD6 gene. x = nonsense mutation, □ = missense mutation.

MODEL OF THE *RAD6* GENE



of these functions; the product functions for induced mutagenesis and sporulation, but not for non-mutagenic repair, conferring extreme sensitivity to DNA damaging agents on rad6-4 strains. Although there is no direct evidence indicating the functions carried out by the RAD6 gene product, its involvement in such a diverse set of processes suggests that it may be a regulatory molecule rather than a enzyme.

This paper is based on work performed under contract with The U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics and has been assigned Report No. UR-3490-1677.

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CHAPTER TWO

LOSS OF PHOTOREVERSIBILITY IN YEAST DEPENDENT ON *DE NOVO* PROTEIN SYNTHESIS

by

Jean Douthwright-Fasse, Christopher W. Lawrence

and William Farland

(Submitted to: Mutation Research)

ABSTRACT

The loss of photoreversibility (LOP) of ultraviolet induced mutations to arginine independence in an excision defective strain of Saccharomyces cerevisiae carrying arg4-17 is dependent upon de novo protein synthesis. This post UV protein synthesis causes pyrimidine dimers to become inaccessible to the photoreactivating enzyme in some unknown manner. LOP begins immediately after UV irradiation, before semiconservative DNA synthesis takes place, and is complete after four hours in growth medium. There is no evidence indicating whether the normal function of the protein is involved in excision repair, or in one of the two repair processes believed to be inducible; induced mutagenesis (12) or recombinational repair (4).

INTRODUCTION

Photoreversibility has been used as a probe to investigate the initial events leading to recovery from lethal UV-damage or UV mutagenesis because the photoreactivating enzyme acts with high specificity to split cyclobutyl pyrimidine dimers (24), and because of the ease with which photoreactivation can be applied, especially since there is no nonenzymatic photoreversal in the yeast. As a result of experiments using photoreversibility of induced prototrophs in wild type strains, Witkin (31) proposed that there was "dark repair" of dimers in E. coli caused by UV irradiation. Nishioka and Doudney (17) showed that loss of photoreversibility (LOP) of UV induced mutation to streptomycin resistance in excision proficient E. coli strains did not require either protein synthesis or DNA replication, but did require energy. However, in excision deficient mutant strains the loss of photoreactivation is delayed and was dependent on both protein and DNA synthesis. The mutations presumably arise during the repair of daughter strand gaps opposite dimers in the newly formed DNA strands (23).

We have used photoreactivation in a similar manner to examine the early events leading to the production of UV induced mutations in the yeast, Saccharomyces cerevisiae. We find that photoreversibility in yeast is lost by a mechanism that is different from those in E. coli. LOP begins immediately after UV

irradiation even in excision deficient strains, is dependent on protein synthesis, but occurs before and independently of semiconservative DNA synthesis in excision defective strains. These data suggest that a protein is synthesized after UV irradiation causing the pyrimidine dimers to become inaccessible to the photoreactivating enzyme in some unknown manner. The normal function of this protein is unknown but may be involved in excision repair or in either of the two processes thought to be inducible, UV induced recombination (4) or mutagenesis (12).

MATERIALS AND METHODS

Strains: All strains were constructed to contain the highly UV revertible allele arg4-17 (13). The source of excision defective and temperature sensitive mutations are shown in Table 1.

Media: Strains were grown for three days (to stationary phase) at 30°C, or at 23°C for temperature sensitive mutant strains, in liquid YPD (1% Bacto yeast extract, 2% Bacto-peptone and 2% dextrose) and appropriate dilutions of washed cells plated onto SD (0.17% Difco yeast base without ammonium sulfate and amino acids, 0.5% ammonium sulfate, 2% dextrose, 1.5% Difco agar Noble, supplemented with all nutrilites and 0.2 mg/liter arginine) for arg4-17 revertants (9) and onto fully supplemented SD for corresponding estimates of viability. Ten plates were used for each dose and dilution for scoring arg4-17 revertants and four plates for viability estimates.

Inhibitors: Cycloheximide (10 µg/ml) (Sigma Chemical Co.) and hydroxyurea (0.1M) (Sigma Chemical Co.) were added to liquid SD medium. Cultures at a concentration of 1 X 10^7 cells/ml were treated with inhibitors by holding them in liquid SD medium in the presence of the drug for 20 minutes prior to UV irradiation and for appropriate times after UV irradiation. They were then washed three times before plating and photoreactivation. Non-growth liquid media was synthetic media lacking dextrose or a 0.1 M potassium phosphate buffer.

TABLE 1

STRAINS

Allele	source	isolated by
rad1-1	B.S. Cox	S. Nakai and S. Mutsumoto (16)
rad2-5	"	B.S. Cox and J.M. Parry (1)
rad3-2	"	"
rad4-4	"	"
rad10-2	"	"
rad16-2	"	"
rad1-2	"	R. Snow (26)
cdc21-1	L. Prakash	L. Hartwell (6)
prt3-1	Yeast Stock	L. Hartwell (8)

Irradiation and photoreactivation: Germicidal UV irradiation was carried out as reported in earlier publications (12) and photoreactivation treatments performed in an incubator illuminated by F20T12/BLB black light fluorescent tubes (General Electric). Radiation from these tubes (300 to 400 nm, peak 365 nm) was filtered through a hammered glass diffuser 4 mm thick and through a plastic Petri dish lid to remove shorter wavelengths only. All photoreactivation exposures lasted for 30 minutes which gives maximal photoreactivation without affecting survival. Treatments were carried out at 30°C unless the strains were temperature sensitive, in which case they were treated at 37°, the nonpermissive temperature, or 23°, the permissive temperature. All strains used in the experiments were selected to show at least 60% photoreactivation of induced reversion when treated immediately after UV irradiaton. The non-photoreactivable sector after immediate photoreactivation was subtracted from the data, and the results expressed as the percent of the initial amount of photoreactivation that was lost. All germicidal irradiations were carried out at room temperature and under illumination solely from "gold" fluorescent lamps to avoid unintentional photoreactivation. In experiments examining the effect of inhibitors of growth conditions, germicidal UV-irradiations were given to cells suspended in liquid medium. In these cases, a suspension of 1×10^7 cells/milliliter was constantly stirred during irradiation and immediately photoreactivated in liquid medium or washed, plated and photoreactivated at the appropriate time.

Fluorometric DNA assay: The amount of DNA in irradiated and control cultures was assayed by the method of Kissane and Robins (11) as modified by Doi and Doi (2) for yeast cells, using an Aminco fluorimeter. 1.0N HC1 was substituted for 0.6N perchloric acid as suggested by Fast (Dale Fast, personal communication).

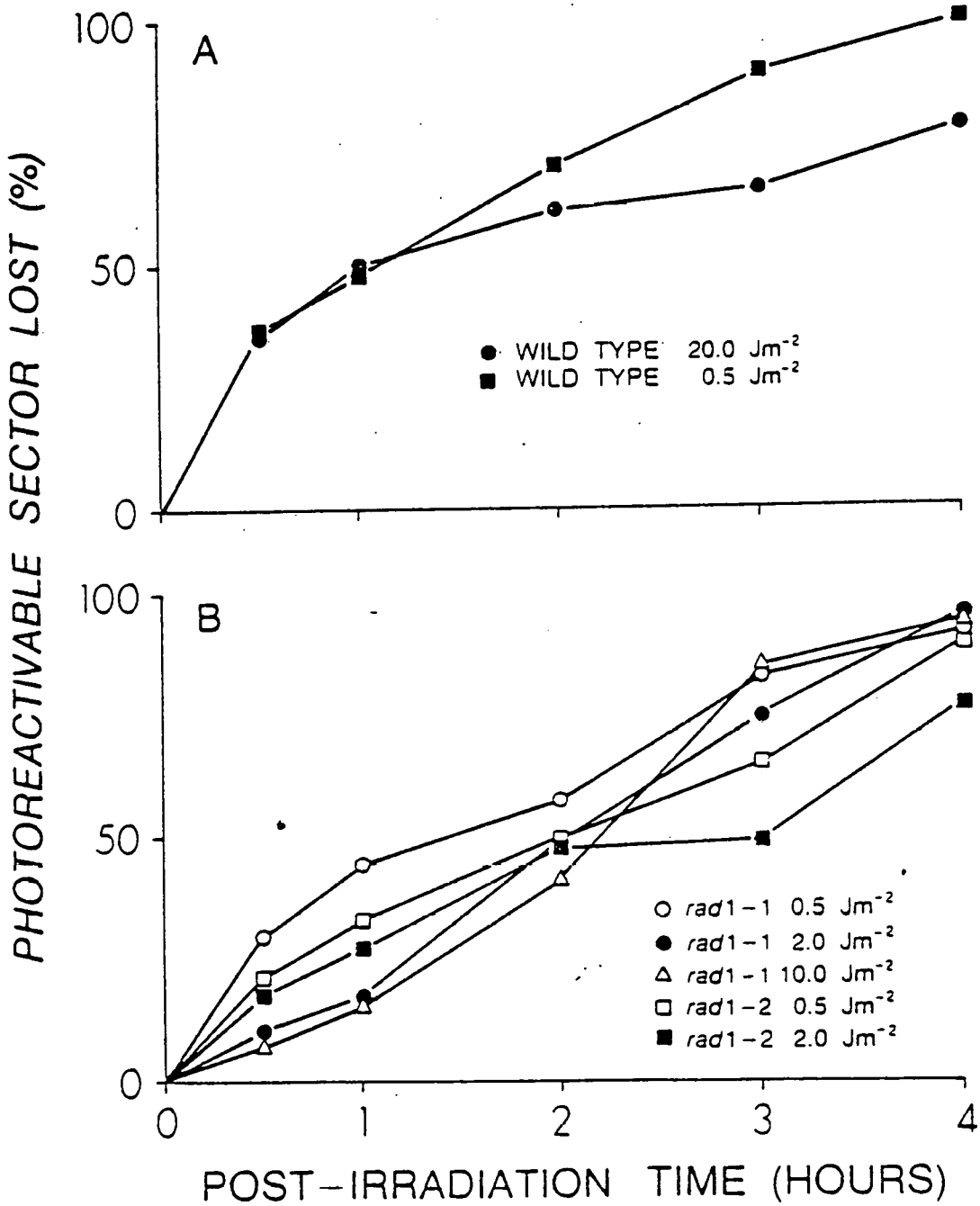
Photoreactivating enzyme assay. The enzyme assay used was the rapid dimer assay method of Sutherland and Chamberlain (27), and yeast cell extracts were obtained after treatment with zymolase.

RESULTS

Loss of photoreversibility in excision defective strains: In wild type E. coli loss of photoreversibility (LOP) of UV induced mutations to streptomycin resistance occurs with the same kinetics as excision repair, and in excision defective strains is delayed until DNA synthesis (17). In yeast the time course and conditions for LOP are different. LOP of mutation induction at the arg4-17 site is similar in both wild type and rad1-1 (16,29) and rad1-2 (29) excision defective strains, beginning immediately after UV irradiation and reaching a maximum by about four hours after irradiation (Figures 1A and 1B). Each of these cultures was derived from stationary phase cells diluted into fresh medium, so that all cells are G_1 at the beginning of the experiment (7). LOP is not unique to rad1 mutant strains. Other excision defective mutants carrying rad2-5, (5,22) rad3-2, (16,19) rad4-4, (5,19) rad7-1, (Prakash, personal communication) rad10-2, and rad16-1 (21) also exhibit LOP (Douthwright-Fasse, unpublished results). Therefore, LOP does not appear to depend upon normal excision repair, or at least on those activities that are missing in these excision deficient mutants.

The shape of the curve is not dose dependent, as seen in Figures 1A and 1B. This observation, together with the extreme sensitivity of the rad1-1 and rad1-2 strains, and the similarity in the LOP kinetics of wild type and excision defective strains makes

FIGURE 1 Average LOP after UV irradiation with the indicated dose in wild type strains (JF101, JF234), rad1-1 strains (JF241, JF242), and rad1-2 strains (JF126, JF184).


A
B
100
50
0
WILD TYPE 20.0 Jm⁻²
WILD TYPE 0.5 Jm⁻²
rad1-1 0.5 Jm⁻²
rad1-1 2.0 Jm⁻²
rad1-1 10.0 Jm⁻²
rad1-2 0.5 Jm⁻²
rad1-2 2.0 Jm⁻²
0
1
2
3
4
PHOTOREACTIVABLE SECTOR LOST (%)
POST-IRRADIATION TIME (HOURS)

it unlikely that the results are due to the "leakiness" of the rad1 alleles. The wild type strains were treated with 0.5 Jm^{-2} and 20.0 Jm^{-2} giving 100% and 90% survival, while the excision defective strains were treated with 0.5 Jm^{-2} and 2.0 Jm^{-2} giving 80-95% and 10-20% survival. These results show that LOP occurs at the same rate regardless of both survival and the number of dimers in the genome, an observation made by other workers as well (3).

All of the following experiments were carried out using a low fluence that gave high survival (80-90%), to minimize survival artifacts. LOP experiments were carried out in strains carrying the rad1-1 mutant allele since they are among the most UV sensitive excision defective strains probably as a consequence of the rad1-1 allele being non-leaky.

LOP occurs in G1 cells: Unlike excision defective strains of E. coli, in which LOP is delayed until semiconservative DNA synthesis begins (17), the onset of LOP occurs immediately in rad1-1 strains of yeast, suggesting that it takes place in G1 cells. This was confirmed by experiments of three kinds. First the amount of DNA present in these cultures was determined at intervals up to twelve hours after dilution of the stationary phase cells into fresh growth medium. (Figure 3A). DNA synthesis begins at about 2-1/2 hours after UV irradiation in these cultures and budding, which Hartwell (7) has shown occurs just prior to DNA synthesis, begins between 2 hours and 2-1/2 hours in the same cultures (Douthwright-Fasse, unpublished results). About half of the

FIGURE 2 Average LOP after UV irradiation with 20.0 Jm^{-2} of *rad*1-1 strains (JF237, JF246) held in growth conditions and non-growth conditions, *rad*1-1 strains (JF237, JF241) after UV irradiation with 20.0 Jm^{-2} in the presence of 10 µg/ml cycloheximide, and *rad*1-1 *prt*3 strains (JF194, JF195) after UV irradiation with 2.0 Jm^{-2} and held at 23° and 37°.

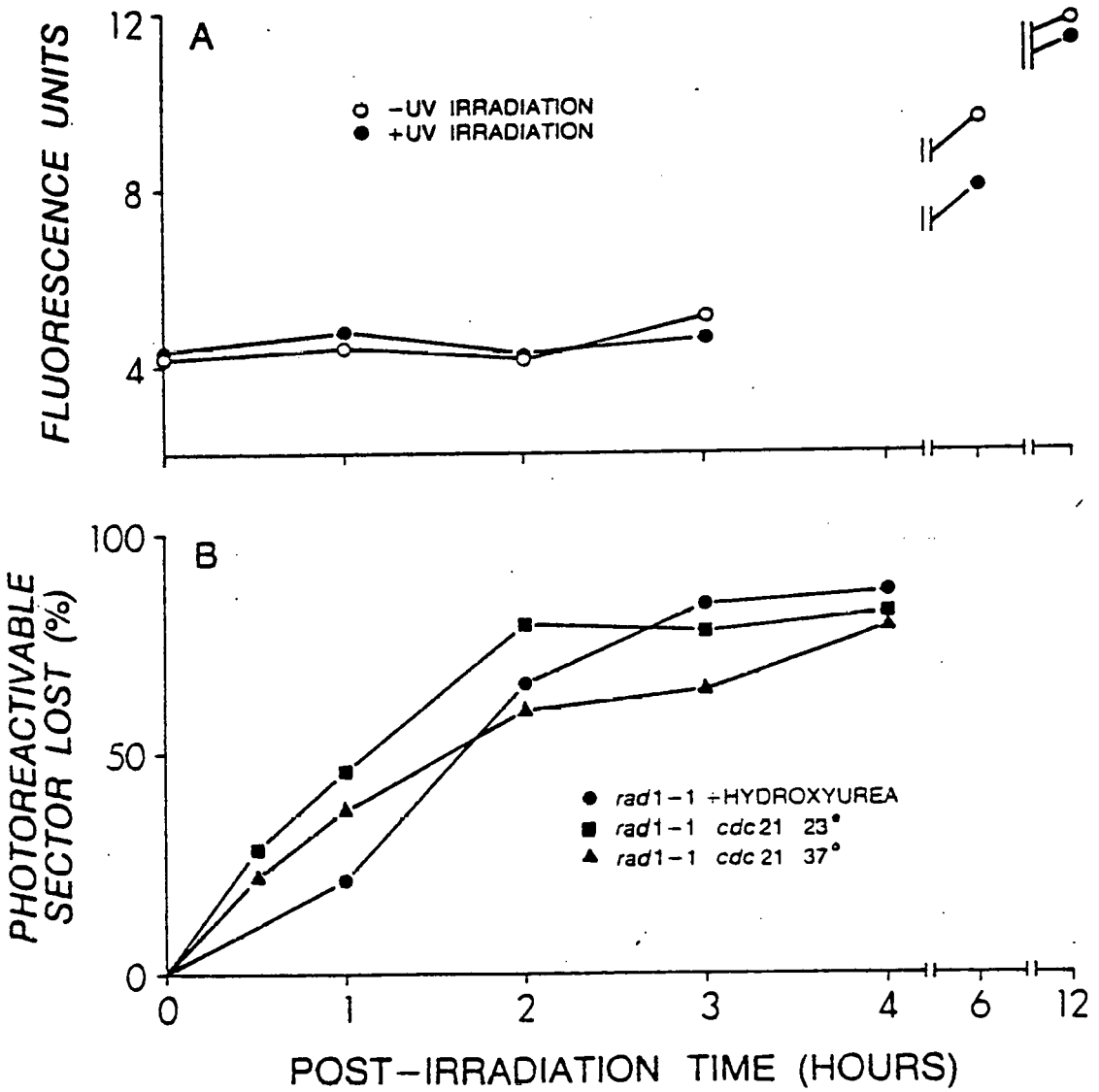
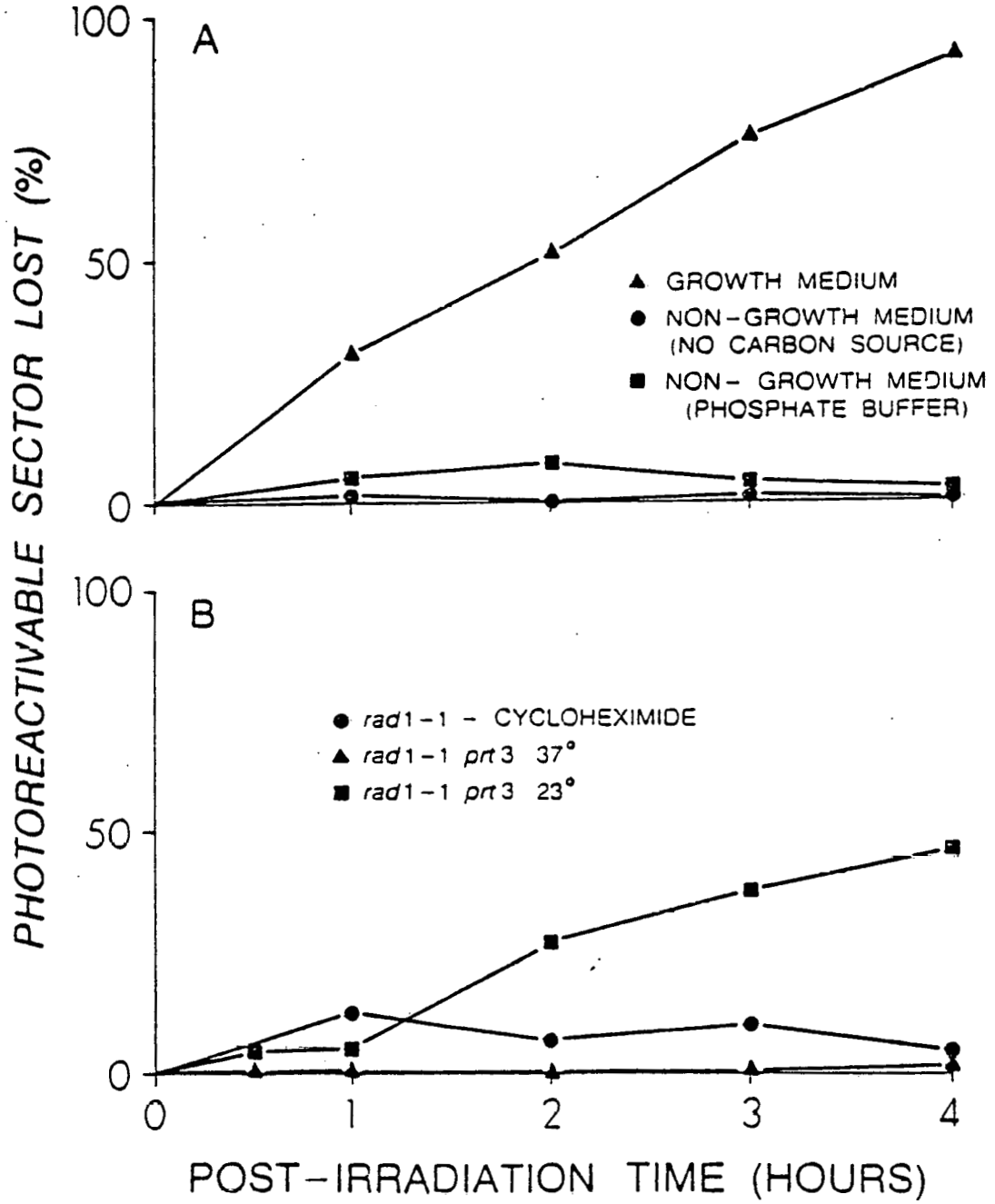

A
12
8
4
FLUORESCENCE UNITS
○ −UV IRRADIATION
● +UV IRRADIATION
B
100
50
0
PHOTOREACTIVABLE SECTOR LOST (%)
● rad1−1 +HYDROXYUREA
■ rad1−1 cdc21 23°
▲ rad1−1 cdc21 37°
0
1
2
3
4
6
12
POST−IRRADIATION TIME (HOURS)

FIGURE 3 A. Amount of DNA in irradiated and unirradiated rad1-1 strains (JF241, JF242) held in growth medium. The amount of DNA was determined fluorometrically and expressed as fluorescence units and is the average of one run with each strain.

B. Average LOP of rad1-1 strains (JF237, JF241) after UV irradiation with 20.0 Jm^{-2} in the presence of 0.1 M hydroxyurea, rad1-1 cdc21 strains (JF249, JF250) after UR irradiation with 2.0 Jm^{-2} and held at 23° and 37°.


100
50
0
A
GROWTH MEDIUM
NON-GROWTH MEDIUM
(NO CARBON SOURCE)
NON- GROWTH MEDIUM
(PHOSPHATE BUFFER)
100
50
0
B
rad1-1 - CYCLOHEXIMIDE
rad1-1 prt3 37°
rad1-1 prt3 23°
0
1
2
3
4
PHOTOREACTIVABLE SECTOR LOST (%)
POST-IRRADIATION TIME (HOURS)

maximum LOP is over at two hours (see Figure 1) in both wild type and excision defective strains. The control non-irradiated culture begins DNA synthesis at about two hours after transfer to growth medium.

Second, photoreversibility is also lost in excision defective cells in which DNA synthesis is blocked by the temperature sensitive mutation cdc21 or inhibited by hydroxyurea. Figure 3B shows the LOP results with rad1-1 strains carrying cdc21, a temperature sensitive mutation deficient in DNA elongation at 37°, and relatively normal at 23°. LOP occurs at both the permissive and nonpermissive temperatures in these strains. Figure 3B also shows that LOP can occur in rad1-1 strains grown in the presence of the DNA synthesis inhibitor hydroxyurea (0.1M). For unknown reasons, photoreactivation in the cells treated with hydroxyurea was only about 40-50% effective, even in the best rad1-1 strains. The data from the rad1-1 cdc21 strains and hydroxyurea treated rad1-1 strains nevertheless suggests that the LOP process is not dependent on semiconservative DNA synthesis.

Dependence of LOP on growth: Parry and Parry (18) have shown that photoreactivation of lethal damage in excision defective strains of yeast, including those carrying a radl mutation, was not lost when these strains were held in saline, that is under conditions in which they cannot grow. Wild type strains, however, do slowly lose the ability to photoreactivate such damage when held in saline.

Figure 2A shows that photoreversibility of mutation induction is also maintained in excision defective strains held under nongrowth conditions, although when held in growth conditions those same strains show LOP. Photoreversibility of mutation induction at the arg4-17 site is not lost in cultures held either in 0.1M phosphate buffer or minimal SD medium lacking dextrose. A ten fold higher fluence of germicidal UV was used for the experiments in liquid medium than in experiments carried out on solid medium because the liquid medium strongly absorbs UV and also because of the shielding effect of cells at the concentrations used (Douthwright-Fasse, unpublished results). The two treatments nevertheless give comparable survivals (80-90%).

LOP depends on protein synthesis: Our data show that loss of photoreversibility starts immediately after UV irradiation in rad1-1 strains of yeast, occurs independently of semiconservative DNA synthesis, but is dependent upon growth conditions. This suggests that LOP may depend on protein synthesis, and we have examined this possibility in two ways; by inhibiting protein synthesis with the temperature sensitive mutation prt3-1 and also by using cyclohexamide. The results are plotted in Figure 2B. Relatively synchronous G1 cultures of prt3-1 rad1-1 strains grown at 23° were spread on plates prewarmed to either 23° or 37°, and held for twenty minutes at these temperatures prior to irradiation. The prt3-1 mutant allele is defective in the elongation of polypeptide chains, and exhibits a rapid cessation of

protein synthesis within twenty minutes after a shift to the restrictive temperature, 37° (8). LOP occurs in these strains when grown at the permissive temperature, but not at 37°. At 23° LOP does not occur as quickly as in PRT3$^+$ strains at 30° (see Figure 1B), probably because the prt3 strains are still partially mutant at the permissive temperature of 23°. PRT3$^+$ strains show approximately normal LOP kinetics at 23° (unpublished data). Also shown in Figure 2B are LOP results for rad1-1 strains (a cell concentration of 1 x 10^7 cells/milliliter) treated with 10 µg/ml cycloheximide in liquid medium for twenty minutes prior to UV irradiation. These cultures exhibit no LOP. Rad1-1 strains are more sensitive to cycloheximide than wild-type strains (66% and 90% survival respectively for rad1-1 and wild type strains after four hours of 10 µg/ml of cycloheximide, with no UV irradiation). Both the prt3 and cycloheximide results suggest that LOP is dependent upon de novo protein synthesis after UV irradiation.

Activity of the photoreactivating enzyme: The previous results indicate that photoreversibility is lost under growth conditions and that the loss is dependent on de novo protein synthesis. The consequence of this protein synthesis appears to be that cyclobutyl dipyrimidines become inaccessible to the photoreactivating enzyme, either because of induced changes in the dimer or because of conformational changes in the chromatin, rather than the degradation or inhibition of the photoreactivating enzyme. Two sets of experiments were carried out which indicate that activity

of the photoreactivating enzyme is not lost after UV irradiation. The results for the first, an in vivo test for photoreactivating enzyme activity, are shown in Table 2. Wild-type, rad1-1 and rad1-2 strains growing on plates were UV irradiated and challenged with photoreactivation at the indicated times. All strains can, as expected, photoreactivate damage introduced immediately after transfer of these relatively synchronous G1 cultures to growth medium (zero treatment time - 42 - 81% PR) and also when damage is introduced four hours after transfer (36 - 80% PR). They can, however, also photoreverse newly introduced pyrimidine dimers four hours after an initial exposure to UV followed by transfer to growth media and incubation in the dark (73%, 59% and 86%). Calculations which lead to this conclusion are shown in TABLE 2. To determine the photoreversibility of newly introduced pyrimidine dimers in dark held cultures that had been previously irradiated, two calculations are needed. The first is the number of UV induced revertants caused by irradiation at four hours in a previously irradiated cell population (treatment E - treatment A.) To get this number, the number of UV induced revertants caused by the initial irradiation treatment A must be subtracted from the total number of UV induced revertants from the combined treatment E. The calculation seems valid since all equivalent total UV fluences yield similar reversion frequencies, i.e., $A + C \simeq E$. The wild type strain allows excision repair to remove some of the pyrimidine dimers, and the repair is probably more efficient at the lower

TABLE 2

PHOTOREACTIVATION IN RE-IRRADIATED CELLS

	Treatment				wild type * (JF 101)	radl-1** (JF 184)	rad1-2** (JF 237)
	0		4 hours		arg4-17 revertants/ 10^8 survivors	arg4-17 revertants/ 10^8 survivors	arg4-17 revertants/ 10^8 survivors
	UV	PR	UV	PR			
A	1	-	0	-	160	75	599
B	1	+	0	-	30	27	240
C	0	-	1	-	230	11	586
D	0	-	1	+	122	4	310
E	1	-	1	-	556	97	924
F	1	-	1	+	225	87	619
G	1	-	0	+	119	78	574
H	2	-	0	-	892	187	1605
I	2	+	0	-	182	53	924
J	0	-	2	-	976	192	1276
K	0	-	2	+	413	38	815

% PR at zero time 100-(B/A), 100-(I/H)	81%, 80%	64%, 72%	60%, 42%
% PR at four hours 100-(D/C), 100-(K/J)	47%, 58%	64%, 80%	47%, 36%
% PR (of previously irradiated culture) of newly introduced dimers at four hours 100-(F-G)/(E-A)	73%	59%	86%

* UV fluences 1 = 10.0 Jm^{-2}
2 = 20.0 Jm^{-2}

** UV fluences 1 = 1.0 Jm^{-2}
2 = 2.0 Jm^{-2}

fluence (10.0 Jm^{-2}) accounting for the large numbers of revertants at the 20.0 Jm^{-2} dose. The higher fluences were used for the wild type strain to give survival similar to that in the excision defective strains. The difference in reversion frequency between the two excision defective strains is probably due to an effect of cell density on UV dose in these particular rad1-1 experiments, not an effect of the allele. Split dose recovery (10) for reversion is exhibited by all the strains. This can be seen by comparing the results of treatments E and H. Treatment H, the same total UV fluence as the split dose of treatment E, yields more revertants indicating that the four hour incubtion period between UV irradiation in treatment E allows recovery from DNA damage.

The activity of the photoreactivating enzyme after various combinations of UV irradiation and photoreactivation treatments of an initially synchronous G1 culture of the excision defective strain JF237 held in growth medium is shown in TABLE 3. All the samples exhibit active photoreactivating enzyme. The result of samples 3 and 4 are the most important in supporting the proposed model responsible for LOP. These samples are irradiated at zero time, held for four hours in growth medium, and photoreactivated or50ot photoreactivated. Both of these samples exhibit active photoreactivating enzyme. Therefore the enzyme although unable to carry out photoreactivation under these conditions is still functional. The control, untreated culture (sample 5) exhibits the lowest level of enzyme activity for unknown reasons. In

TABLE 3

SPECIFIC ACTIVITY OF PHOTOREACTIVATING ENZYME

(JF237)

	time of treatment (hours)***					
	UV*	0 PR	4 PR	amount extract	activity**	average activity
1	+	-	-	50 µl	54.4	51.35
				100 µl	48.3	
2	+	+	-	50 µl	79.1	82.75
				100 µl	86.4	
3	+	-	-	50 µl	44.7	49.60
				100 µl	54.5	
4	+	-	+	50 µl	72.3	70.00
				100 µl	67.7	
5	-	-	-	50 µl	38.3	33.55
				100 µl	28.8	

* 10 Jm^{-2}

** expressed as pM DNA photoreactivated/hour photoreactivation/ mg protein

*** Samples 1, 2 and 5 were collected at zero time while samples 3 and 4 were held in minimal growth medium for four hours before being collected.

conclusion, it seems likely the photoreactivating enzyme is still active and not degraded in irradiated cell cultures held for four hours under growth conditions.

DISCUSSION

Our results indicate that protein synthesis immediately after UV irradiation acts in some unknown manner to make pyrimidine dimers inaccessible to the photoreactivating enzyme. The dimers may be altered in some way, bound by a protein(s) or the conformation of a specific area of the chromatin might be affected, creating inappropriate binding conditions for the enzyme. Although it cannot be excluded that such changes are not related to repair, it seems more likely that they are an integral part of one of the several repair processes that take place in yeast. The loss of photoreversibility as assayed by reversion of the arg4-17 allele in yeast is not dependent upon semiconservative DNA synthesis, however, as is reversion to streptomycin resistance in excision defective E. coli (17). Such results are not restricted to reversion at the arg4-17 site since the lys2-1, cyc1-9, and cyc1-115 alleles also exhibit LOP beginning immediately after UV irradiation in excision deficient strains (Douthwright-Fasse, unpublished results). Moreover, mutations are induced effectively by dimers located far from the site of reversion (Lawrence and Christensen, manuscript in preparation), so it is unlikely that these results reflect the ability of the photoreactivating enzyme to split dimers located in only one small region of the genome. The protein responsible for LOP has not been identified or isolated.

It seems likely that the LOP is a repair dependent process since there is probably an effect upon the pyrimidine dimers by the de novo protein synthesis. Any protein present prior to UV irradiation is unable to function allowing LOP as is exhibited utilizing the prt3 mutant and cycloheximide. Therefore, the LOP process is apparently inducible, but how and when the protein acts in the DNA repair process is not known.

Since the data show that LOP begins immediately in these relatively synchronous G1 cells, the process may be involved in excision repair. Even "abnormal" or mutant excision activities function for LOP, but not for excision repair. Although Prakash has shown that rad1-2 (20), rad3, rad4, (19), rad10 and rad16 (21) are defective in dimer excision little is known about these mutants biochemically. Rad1-2 strains show no evidence of removal of pyrimidine dimers after treatment with low UV fluences and four hours incubation in growth medium, in contrast to wild type strains which remove virtually all of the induced dimers during this period (20). The removal was assayed by treatment with the UV endonuclease causing single strand breaks in DNA containing pyrimidine dimers. These results suggest that pyrimidine dimers, the substrate for endonuclease activity, are still present after a four hour period of growth. Resnick and Setlow (22) have shown similar results in rad2-17 strains utilizing a competition method for photoreactivating enzyme between UV irradiated calf thymus DNA and the crude extracts of the UV irradiated yeast cells. Our data

indicate that an alteration of some type occurs leading to an inaccessibility of the photoreactivating enzyme to the dimer beginning immediately prior to incision. The LOP results may indicate a process which is initiated in G1 and not completed until the G2 period of the cell cycle. The de novo protein synthesis necessary for LOP may be a factor (5) required for excision repair. Mortelmans et al. (15) suggest that Xeroderma pigmentosum cells, which are excision defective lack such an additional or alternative factor(s) rather than the enzymes required for excision repair. Mutation expression, however, does not occur in these strains until after semiconservative DNA synthesis occurs according to James et al. (9), although our results indicate that photoreversibility of dimers is lost earlier.

LOP or de novo protein synthesis may instead function as a signal to one or more of the repair activities that are thought to be inducible and occur after DNA synthesis; such as recombination (5), or error prone repair (12). Fabre and Roman (5) propose that one effect of radiation is to release or to promote the synthesis of synthesis some factor(s) to initiate recombination resulting in an increase in the proportion of competent cells able to recombine. Yeast possess a single activable error prone cluster of activities for the repair of UV damage (12). This repair can be mediated across the nuclear membrane (Lawrence and Christensen, in preparation). Nothing is known about how this repair is activated, and the process could also be a candidate for activation by the de novo protein synthesis required for LOP.

During the progress of this study, work along similar lines was published, carried out in Chlamydomonas reinhardi (24). Loss of photoreversibility in an excision defective mutant strain was determined either at zero time or at 24 hours after UV irradiation by measuring the susceptibility of pyrimidine dimers in the DNA to hydrolysis by UV specific endonuclease. This process is independent of protein synthesis, and thought to be due to inactivation of the photoreactivating enzyme in irradiated cells after twenty four hours of dark incubation. We see no inactivation of the enzyme in irradiated excision defective yeast strains held in the dark for four hours. The LOP occurring in our system seems to be due to the inaccessibility of the dimer by the enzyme, not the degradation or inactivation of the enzyme. Other workers have shown the PR enzyme activity in Chlamydomonas is lost after 365 nm radiation (25), and have proposed that the enzyme is inactivated by irradiation at this wavelength. We see no evidence of inactivation of the yeast enzyme due to treatment of this kind. The two systems, yeast and Chlamydomonas, seem to have different repair activities to deal with DNA damage.

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DISCUSSION

These studies have examined in two diverse manners the DNA repair processes of the eucaryotic yeast Saccharomyces cerevisiae, especially the possibility of an enzymatically induced or regulated mechanism of repair. The first study utilized a new allele, rad6-4, of the RAD6 gene. The characterization of strains carrying the rad6-4 allele suggest that the processes which are dependent upon the RAD6 gene product are of at least two kinds; the first being non-mutagenic and important for survival, while the latter is mutagenic and relatively unimportant for survival, although necessary for sporulation. The second area of study utilizes the loss of photoreversibility (LOP), as assayed by the reversion of the highly UV-revertible arg4-17 allele, to probe the events occurring during the first cell cycle after DNA damage. The results indicate the likelihood that de novo protein synthesis, which is required for LOP, acts in some manner upon the pyrimidine dimers, or on the structure of the damaged chromatin creating inappropriate binding conditions for the photoreactivating enzyme during the G1 period of the cell cycle. This discussion will relate these results to those of other workers in DNA repair and mutagenesis.

Other studies also yield supportive information regarding the existence of several branches of RAD6 dependent activities, an idea which is given substance by the present studies on the new rad6-4

allele. Further evidence comes from the observations of the metabolic suppressor mutations SRS2, on all three rad6 mutant strains. These have the proficiency to suppress the extreme UV sensitivity or error free repair capacity, of rad6 strains (Lawrence and Christensen, in press). The suppressors have no effect on either the mutational or sporulation deficiency of rad6 mutants. Thus the two activities controlled by the RAD6 gene can be separated mutationally utilizing either the rad6-4 or SRS2 mutations, suggesting that these are enzymatically separate processes or branches to repair DNA damage.

In view of two enzymatically separate branches of DNA repair possibly regulated by the RAD6 gene, it seems worthwhile to examine how other mutations in the RAD6 epistasis group, rad8, rad9, rad15, rad18 (Lawrence and Christensen, 1976) and the rev mutations, rev1, rev2 and rev3 (Lemontt, 1971) are involved in the two RAD6 controlled functions. The most information comes from studies of the rad18 mutation. Like rad6 strains they are extremely sensitive to UV irradiation, and the sensitivity can be suppressed by SRS2. They are also sensitive to ionizing radiation (Lawrence et al., 1974; von Borstel et al., 1971) though not to the same extent as rad6 strains, and to chemical mutagens (Prakash, 1976) and trimethoprim. In contrast to rad6 strains, rad18 strains allow UV-induced mutagenesis at nearly normal levels (Lawrence, personal communication) and also allow normal levels of sporulation. Earlier data (Lawrence et al., 1974) indicating that

rad18-2 strains were deficient in UV-induced mutagenesis have not proven typical. These phenotypes suggest that the RAD18 gene product is associated with the error free RAD6 dependent function, but not the mutagenic or error prone repair function.

Since the SRS2 mutation has the capacity to suppress the sensitivity of rad6 and rad18 strains to UV irradiation, but not gamma radiation it seems probable that the error free repair of UV and gamma ray induced DNA damage are controlled by different processes. The properties of two other mutations in the RAD6 epistasis group support this hypothesis. Both rad9 and rad15 mutant strains are extremely sensitive to gamma rays. The rad9 strains are as sensitive to gamma rays as rad6 mutant strains (McKee and Lawrence, in preparation), while both rad9 and rad15 mutant strains are somewhat sensitive to UV irradiation (Cox and Parry, 1968; Lawrence et al., 1974). Both allow sporulation but neither are sensitive to trimethoprim. Rad9 mutant strains allow normal gamma ray mutagenesis at the cyc1-9 site in the four strains examined under various physiological conditions (McKee and Lawrence, in press), normal UV induced mutagenesis at the cyc1-131 site, and UV induced mutagenesis only at low doses in three strains for the cyc1-9 site. Mutagenesis in these strains was not exhibited at higher doses where artifacts are more common (Lawrence et al., 1974).

Therefore there seem to be at least two error free RAD6 dependent repair processes for UV and gamma ray induced

mutagenesis. The first RAD6 dependent repair process requires the RAD18 gene and is principally involved with the repair of UV induced DNA damage as well as trimethoprim resistance; the second requires RAD9 and RAD15 genes which seem to be principally responsible for the repair of gamma ray induced damage. Rad18 mutant strains, however, are somewhat gamma sensitive and rad9 and rad15 strains are somewhat UV sensitive. These sensitivities exhibited by the rad9, rad15, and rad18 mutant strains may be due to the ability of these gene products in wild type strains to act upon a variety of DNA lesions induced by different mutagens, but with a higher specificity for certain types of lesions.

There is less data available concerning rad8 mutant strains. Results for two mutant rad8 strains (Lawrence and Christensen, 1976) show substantially reduced UV induced mutagenesis at the cyc1-9 site, but only a small effect on survival. Therefore the RAD8 gene is probably involved in the error prone repair dependent RAD6 process.

The three REV alleles, rev1, rev2, and rev3 pose somewhat of a paradox because they are sensitive to UV irradiation, even though they are the least UV sensitive mutants in the RAD6 epistasis group. The rev mutant strains are deficient in specific types of UV induced repair, one of the major functions controlled by the RAD6 gene. It might be expected that the rev mutant strains would therefore be almost as proficient as wild type strains in promoting survival after UV irradiation, the second major function controlled

by the RAD6 gene, and not sensitive to UV irradiation. Mutant strains carrying these alleles have very specific effects on the sites at which UV induced mutagenesis occurs. The REV1 gene function seems to be required for base pair substitutions rather than additions or deletions after DNA damage, except at the cyc1-131 and cyc1-115 sites (Lawrence and Christensen, 1978a). Whether these results are caused by the genetic nature of these sites, or the specific nucleotide sequence, which has been determined (Stewart et al., 1971; Stewart and Sherman, personal communication), cannot be ascertained with the present data. The evidence for the site specific control of mutagenesis however is clear, although the mechanism seems complex. Rev2 mutant strains are not very sensitive to UV irradiation, and reduce the frequency of UV induced mutagenesis at ochre sites (Lawrence and Christensen, 1978b). REV2 does not seem to be required for reversion of amber, missense, or frameshift mutations. Whether the phenotypes expressed by these mutant strains are a reflection of the normal functions carried out by these genes in wild type strains is not known. One might propose for example that the REV2 gene product may act to stabilize specific areas of the DNA structure for the action of other repair enzymes. Not only are Rev3 mutant strains about as UV sensitive as rev1 and rev2 mutant strains, but they also exhibit large reductions in all types of UV induced mutagenesis. Results with these mutants give the first indications of a hot-spot effect or site specificity of action by various gene products, and suggest that UV mutagenesis may not occur uniformly at all sites.

A brief mention should be made about the DNA replication mutant cdc8. Cdc8 mutant strains are slightly sensitive to UV irradiation. It decreases UV induced mutagenesis at the sites examined (Prakash et al., 1979), and seems to be involved in the error prone branch of RAD6 dependent DNA repair.

The nature of repair pathways or clusters after DNA damage from chemical treatment differs from those of radiation repair. The capacity of mutant strains in the RAD6 epistasis groups to cause reversion at the cyc1-131 site after treatment with a variety of chemicals has been examined (Prakash, 1976). Rad6 mutant strains exhibit a decreased frequency of reversion with all the chemicals utilized (4-nitroquinoline-1-oxide, ethyl-methane sulfonate, and nitrous acid). The other mutant strains of the RAD6 epistasis group; rad9, rad15, rad18 and the three rev mutations, exhibit a significant alteration in induced mutations after treatment with certain chemicals but not others. The results suggest that the RAD6 gene products control the repair activities after chemically induced DNA damage, but that the pathways or clusters of activities are independent of those involved in repair after radiation damage.

E. coli DNA repair systems were the first to be studied in depth, and the most is known about them. Therefore its repair systems have been extensively used to propose models of DNA repair. The role of the recA gene in E. coli is now well

characterized and comparisons of its function to that of the RAD6 gene's function may prove useful. Strains carrying either the recA or rad6 mutant alleles have similarities. They each exhibit the most extreme phenotypes compared to other mutant strains within their epistasis groups such as; sensitivity to UV or ionizing irradiation as well as chemical mutagens. The parallel for the lack of recombination in recA mutant E. coli strains might be the lack of sporulation exhibited in rad6 mutant yeast strains. There is no direct evidence that the RAD6 gene product is involved in a major way in recombination, although it may function in a minor way. The RAD52 gene seems to carry out a major function for the repair of double strand breaks and probably recombination (Resnick and Martin, 1976) in yeast. Game et al. (personal communication) have shown that in mutant rad6 strains recombination cannot occur during the premeiotic DNA synthesis, which takes place before commitment to sporulation. The RAD6 gene is required for mutagenic DNA repair (Lawrence et al., 1974) a process which may also involve recombination. Another parallel is that both the $RecA^+$ and RAD6 gene products are required after DNA damage to promote survival and DNA repair. Furthermore, the $RecA^+$ gene product is known to be inducible as a consequence of DNA damage (Radman, 1975) and the RAD6 gene product may perhaps also be a candidate for inducibility. One might also propose that a recA type of mutation in yeast has not yet been isolated and exists separately from RAD6 to induce these activities.

On the other hand, the rad6 mutant strains of yeast can also be shown to exhibit phenotypes similar to those of lexA mutant strains in E. coli. The lexA gene product is though to act as a repressor at the recA operator site (Gudas and Pardee, 1975), and is derepressed by increased synthesis of the recA gene product after DNA damage. A temperative sensitive mutation of the lexA gene, tsl, causes the lexA repressor to have a decreased affinity for the operator region, thus a decreased affinity for the inducer, and allows constituitive synthesis of the recA gene product. The SRS2 mutation which confers increased UV resistance to rad6 strains could be acting in a similar manner as the tsl mutation in E. coli. LexA mutant strains like rad6 mutant strains, are sensitive to DNA damaging agents, and do not allow UV induced mutagenesis. The lexA product is thought to regulate DNA breakdown by mediation of the $recB^{+}$ $recC^{+}$ product, an ATP-dependent nuclease. (Moody et al., 1973).

Other workers (Lawrence et al., 1974; Fabre and Roman, 1977) have suggested that inducible processes are present in yeast with the capacity to control DNA repair. Yeast possess a single activable error prone repair cluster of activities (Lawrence et al., 1974), which can be mediated across a nuclear membrane (Lawrence and Christensen, in preparation). Nothing is known about how the repair process is activated, and the de novo protein synthesis required for LOP may be a candidate for causing the activation. The final proposed inducible process is the release of

some factor(s) promoting recombination resulting in the increase in the proportion of competent cells able to recombine. Again, the *de novo* protein synthesis required for LOP may act in this manner.

The use of photoreversibility of the highly UV revertible *arg*4-17 allele to probe the first cell cycle after DNA damage suggests that *de novo* protein synthesis acts in some manner upon the damage DNA structure. The result is that inaccessibility of the photoreactivating enzyme to the pyrimidine dimer begins quickly in the G1 period of the cell cycle. Semiconservative DNA synthesis is not required for LOP, although the importance of unscheduled DNA synthesis has not been ruled out.

Although LOP begins immediately during the G1 period of the cell cycle and is completed about four hours after UV irradiation the repair process may not occur until S phase or G2. It is not known when or how the DNA lesion is acted upon to yield a revertant at the site examined. The particular protein(s) which acts during LOP is not known. Mortelsman *et al*. (1976) have suggested that *Xeroderma pigmentosum* cells, which are defective in excision repair, lack such an additional or alternative factor(s) required for excision repair.

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APPENDIX I

ATTEMPTS TO ISOLATE rad6 MUTANTS

BACKGROUND

The RAD6 gene is concerned with a variety of important functions necessary for survival and mutagenesis in Saccharomyces cerevisiae. This organism has three major repair processes to deal with radiation damage (Brendel and Haynes 1972; Cox and Game 1974); one that leads to the excision of damage from DNA (Unrau, Wheatcroft and Cox 1971; Resnick and Setlow 1972; Prakash 1977), a second thought to promote repair of double strand breaks by a recombination dependent mechanism (Resnick and Martin 1976, Ho 1975), and a third concerned with mutagenesis and recovery from damage caused by a variety of agents that is dependent on mechanisms as yet not well understood (Lawrence and Christensen 1976; Prakash 1974; Lemontt 1971).

The RAD6 gene appears to be one of the most important among the group involved in carrying out the third of these processes. Strains carrying mutant alleles of the RAD6 gene exhibit a wide variety of phenotypes; sensitivity to many DNA damaging agents such as radiation and chemicals, lack of mutagenesis induced by such agents, sensitivity to the folate antagonist trimethoprim, and lack of sporulation in homozygous diploids. Strains carrying mutations in other genes that affect the process of mutagenesis and recovery after DNA damage never exhibit all of these phenotypes, and any one

mutant phenotype is rarely as extreme. These properties suggest that the RAD6 gene function is important for a variety of processes, and raises the possibility that the gene is multifunctional.

In order to learn more about the RAD6 gene activity and its function in yeast three different isolation procedures were carried out to search for new rad6 mutations; both conditional (temperature sensitive) and nonconditional mutations. In the presence of trimethoprim, a growth inhibitor for rad6 cells, log phase cells were killed selectively by either treatment with the antifungal antibiotic nyastatin, or through inositol-less death, rad6 strains which are not dividing should survive. The third isolation procedure was screening for super UV sensitive strains after mutagenesis of an excision defective strain with the expectation of enhancement in UV sensitivity in a double mutant with respect to the two single mutants. These and other phenotypes of rad6 strains could be utilized in isolation procedures for new rad6 mutants. Thus, rad6-1 and rad6-3 strains do not sporulate, and are sensitive to treatment with X-rays and MMS. However, since approximately 50 loci code for functions indispensible for sporulation (Esposito et al., 1972) and any one of these may be recovered and the possibility of isolating a rad6 mutant using this phenotype is unlikely. MMS sensitivity has been used successfully to isolate rad6-3 (Prakash and Prakash, 1977) and was later used to isolate rad6-4 (Douthwright-Fasse, Christensen and Lawrence, submitted).

The rad6-1 mutation was isolated after mutagenesis with either UV or EMS and followed by filter pad replica plating in an attempt to obtain as many mutations as possible among the dark repair genes of yeast (Cox and Parry 1968). Game, Little and Haynes (1975) isolated a mutant strain sensitive to trimethoprim, a folate antagonist, while investigating the usefulness of trimethoprim in the selection of dTMP permeable yeast strains. Of the 14 from the 30,000 survivors after X-irradiation, one mutant was found to be sensitive to both ultraviolet light and X-irradiation, and allelic to rad6-1. It was designated rad6-2 but has since been lost. It was found that both rad6-1 and each of the four known alleles of rad18 conferred sensitivity to trimethoprim, but all the other mutant rad strains were trimethoprim resistant like wild type strains.

Trimethoprim sensitivity was used for enrichment in conjunction with two of the isolation procedures. The first was the use of the antifungal antibiotic nyastatin in the presence of trimethoprim. Snow (1966) showed that nyastatin could be used for enrichment and selection of auxotrophic yeast mutants. Nyastatin binds to sterols in the cell membrane and can be used in a manner analagous to pencillin selection widely used in bacteria for the isolations of mutants (Lederberg and Zinder, 1948). The yield of mutants by nyastatin selection in yeast is lower than penicillin selection in E. coli.

The second isolation procedure utilized an inositol requiring mutant of Saccharomyces cerevisiae (Henry and Horowitz, 1975). The

double mutant strain MC3 (a, ino1-13, ino4-8) is very stable and should not revert under normal growth circumstances. Inositol is a major carbohydrate moiety present in a limited number of membrane phospholipids of yeast, and the MC3 auxotroph dies logarithmically when starved for inositol. We expected that only those temperature sensitive mutations which have the effect of rapidly stopping macromolecular synthesis without affecting cell viability would be selected by inositol or nyastatin enrichment in the presence of trimethoprim.

The final isolation procedures was modeled after that of Brown and Kilbey (1970). Super UV sensitive strains were screened for after mutagenesis of a rad1, excision defective strain. RAD6 has been shown to mediate repair in a different repair pathway from excision repair (Cox and Game, 1974) and an enhancement of sensitivity is expected in a double mutant with respect to the two single mutants.

MATERIALS AND METHODS

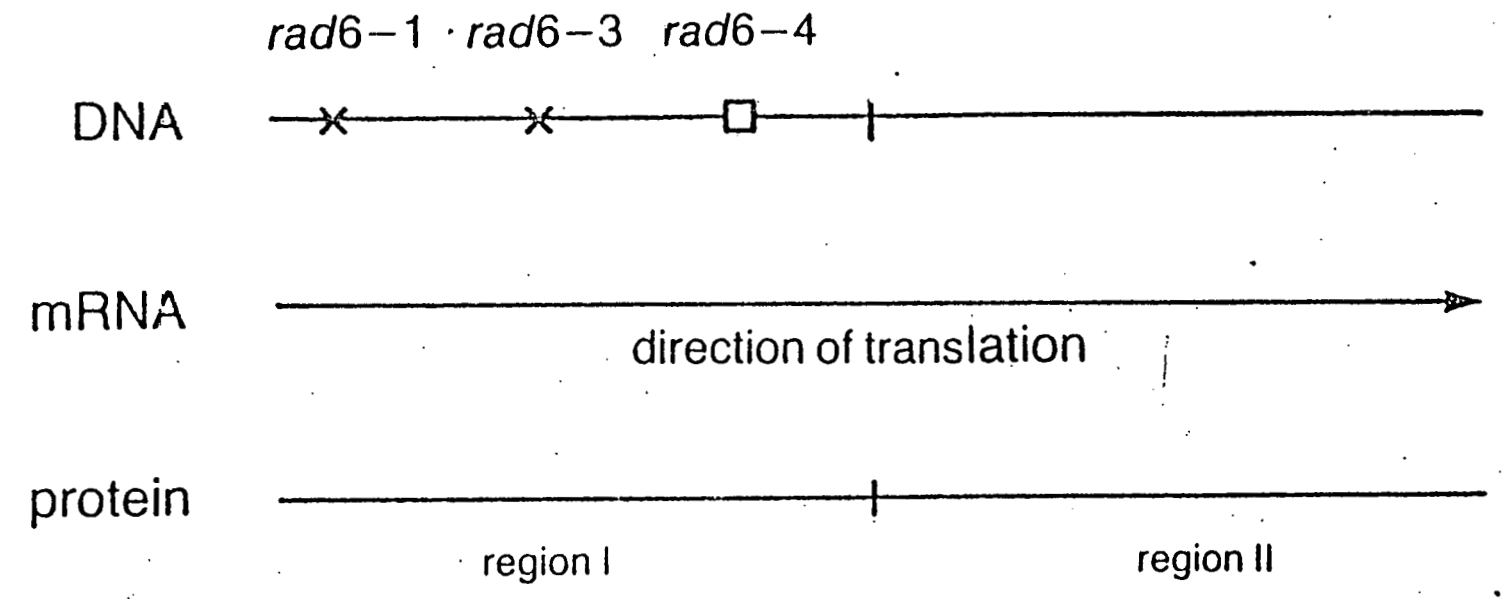
NYASTATIN ENRICHMENT

Strains. CL162-18D (a cyc1-9 arg4-17 leu1-12)

#264 (α ade2-1, from B.S. Cox)

Mutagenesis. One clone of the parental strain was inoculated into 10 mls. of YPD medium (1% Bacto yeast extract, 2% Bacto peptone and 2% dextrose), and grown overnight on a shaker at 30° yielding about 1 X 10^8 cells/ml. The cells were spun, washed, and resuspended in 10 mls. of 0.1M phosphate buffer, pH 7.0. To 2.5 mls. of the suspension was added 50.0 microliter of ethyl methane sulfonate (EMS). The culture was shaken for 40 minutes at 30° and the reaction stopped by adding an equal volume of sterile cold 5% sodium thiosulfate. Survival was 50-60%. The mutagenised culture was spun down and resuspended in 2.0 mls. of sterile distilled water. Then tubes containing 5.0 mls. of liquid YPD were inoculated with 0.1 ml. of the mutagenesised cell suspension and incubated for three days at 30° with shaking to allow for mutant expression. The cells were spun, washed twice, resuspended at approximately 1/4 the cell concentration, about 1 X 10^8 cells/ml, in nitrogen free liquid SD plus amino acid requirements (0.17% Difco yeast base without ammonium sulfate and amino acids, and 2% dextrose) and incubated overnight at 30° on a shaker. 2.0 mls. of the starved culture was washed twice and resuspended in SD medium plus requirements with trimethoprim (0.17% Difco yeast base without ammonium sulfate and amino acids, 0.017% casitone, 2%

MODEL OF THE *RAD6* GENE



dextrose, and trimethoprim 100 µg/ml). Samples were incubated with shaking at both 23° and 36° for about three to four hours until all the cells had buds. An aliquot of cells was plated for estimating the viability. These cultures were than treated with nyastatin (dissolved in dimethyl formamide) at a final concentration of 20 µg/ml and shaken at either 23° or 36° (whatever temperature was used previously for the culture) for about one hour. The cells were washed twice and plated on YPD medium (1% Bacto yeast extract, 2% Bacto peptone, 2% dextrose and 2% Bacto agar) at the appropriate dilutions. The nyastatin gave a four log kill. The colonies were picked and put on a YPD master plate, grown for two days at either 23° or 36° replicated and tested for UV sensitivity and growth inhibition in the presence of trimethoprim at the appropriate temperatures. 369 master plastes from eleven separate experimental runs were examined (253 from isolates at 23° and 116 from isolates at 36° each master plate containing 70 colonies). Of the total 25,830 colonies examined no UV sensitive or trimethoprim sensitive colonies were found.

INOSITOL LESS DEATH ENRICHMENT

Strain. MC3 (a ino1-13 ino4-8)

Mutagenesis. An overnight culture of MC3 was spun, washed and resuspended in phosphate buffer at pH 7.0 and two 2.0ml aliquots taken. One was treated with 30 microliters per ml of EMS for 40 minutes at 30° with shaking, while a control culture was untreated. The reaction was stopped with 9 mls. of 5% thiosulfate. Thiosulfate was added to the control and the viability calculated after appropriate dilutions of treated and nontreated cultures were plated yielding about 60-75% in this strain. The treated suspension was added to 10 mls. of liquid YPD late in the day and grown overnight. The cultures were washed twice, and diluted a hundred fold into inositol less medium (1.7% Difco vitamin free yeast base, 1% dextrose, 0.8% vitamin stock, 4% nutrilite stock and 200 µg/ml trimethoprim) for 24 hours at 30°. The vitamin stock was prepared by dissolving 2mg of vitamin powder (biotin, 2 µg, calcium pantothenate, 400 µg, folic acid, 2 µg, niacin, 400 µg, p-aminobenzioc acid, 200 µg, pyridoxine hydrochloride, 400 µg, riboflavin, 200 µg and thiamine hydrochloride, 400 µg) in 8 mls. of sterile distilled water.

In five of the twelve enrichments carried out using inositol less death a second enrichment like the first was carried out before plating onto YPD medium. Dilutions of the starved cultures were then plated onto YPD medium, survivors picked onto master plates, and spot tested for UV sensitivity. From the 439 masters

colonies), 58 unconditional UV sensitive strains were found. None of these were sensitive to growth inhibition by trimethoprim, and all were complemented for UV sensitivity when crossed to rad6, rad18, and rev3 testers.

TABLE 1

Strains.

Parental CL162-5A (α cyc1-9 arg4-17 leu1-12 rad1-2)

Testers and controls CL166-2C (α)

CL166-3D (a arg4-17 ade2 leu1-12)

CL166-6D (α met1 ade2 lys1)

CL166-8D (α met1 his5 ade2)

CL166-9C (α met1 arg4-17)

CL166-12D (α arg4-17 ade2 leu1-12)

CL166-6C (a rad6-1 his5 lys1)

CL166-23C (a rad6-1 his5 lys1 met1)

CL25-4D (a rad18 lys1 ura4)

CL25-6B (a rad18 his5 ura4)

CL31-3A (a rad18 his5 ura4)

LP204-1B (a rad6-3 ilv3 lys2-1 his1-1 trp2)

CL322-3C (a arg4-17 his1-5 trp2)

SELECTION OF SUPER UV SENSITIVES IN rad1 BACKGROUND

EMS mutagenesis. The parental strain (see TABLE 1) was grown for three days in 10 mls. of liquid YPG (similar to YPD medium but containing 30% V/V glycerol in place of dextrose) at 30°. The culture was spun, washed, and resuspended in 10 mls 0.1M phosphate buffer at pH 7.0. 1.0 ml of the suspension was treated with 30 microliter of EMS for one hour shaking giving 50-60% survival. The reaction was stopped by adding 9.0 mls. of 5% thiosulfate, and the culture was sonicated for 30 seconds to break up clumps of cells. The culture was diluted and plated on YPD medium yielding 40-60 colonies per plate.

UV mutagenesis. The parental strain was grown for three days in 10 mls. of liquid YPD at 30°, washed, and sonicated. About 1 X 10^3 cells were plated onto YPG at medium 30° and irradiated with 4 J/M^2 giving about 10% survival and 50-100 colonies per plate.

Testing. Colonies were picked off onto YPG master plates, grown at 30°, and spot tested for UV sensitivity. Of the 338 master plates tested (23,350 colonies) from eight separate treatments 37 super UV sensitive strains were found, and 27 of these were sensitive to γ-irradiation and to growth inhibition by trimethoprim. The five most sensitive isolates were crossed to a wild type strain (CL332-3C), sporulated and dissected. The segregants were all crossed to rad6 and rad18 tester strains, and the selected diploids were all resistant to UV irradiation. The 10

most sensitive isolates were all crossed to rad6 and rad18 testers and examined for growth inhibition in the presence of trimethoprim and none was found. Therefore, these super UV sensitive strains isolated in these experiments are not rad6 or rad18 mutants. It is likely that they are mutations in the rad52 cluster of repair activities since they are sensitive to γ-irradiation, although complementation with rad52 strains was not performed.

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Appendix II

Table 1

Induced reversion frequency of the arg4-17 allele in haploid and homozygous diploid rad6-3 mutant strains exposed to UV irradiation. Percent survival in parentheses.

Strain	Induced revertants per 10^{-8} survivors (% survival) arg4-17 → ARG4+	
haploids	1.25 Jm^{-2}	2.5 Jm^{-2}
JF152-1C (rad6-3)	0 (71)	0 (54)
JF159-9C (rad6-3)	0 (43)	0 (34)
JF185-2B (WT)	11 (101)	13 (101)
diploids	3.5 Jm^{-2}	7.0 Jm^{-2}
r133 (rad6-3)	0 (17)	0 (5.7)
r135 (rad6-3)	0 (22)	0 (4.0)
r153 (rad6-3)	0 (31)	0 (2.4)
r173 (rad6-3)	0 (73)	0 (9.0)
r191 (rad6-3)	0 (68)	0 (13.0)
r323 (WT)	38 (98)	88 (107)

Table 2

Percent budding cells* from stationary phase rad1-1 (JF242) culture held in growth medium after UV irradiation (20 Jm^{-2} to liquid held cultures).

time (hours)	% budding +UV	% budding -UV
0	0	0
0.5	0	0
1.0	0	0.9
2.0	1.2	9.2
2.5	7.5	29.3
3	31.2	41.8
4	63.0	84.7
6	79.3	91.7

* 2000 cells were counted

Table 3

Loss of photoreactivation of the arg^{4}-17 allele in a $PRT3^{+}$ rad1-1 strain at 23^{o} after 2.0 Jm^{-2} radiation

post irradiation time (hours)	photoreactivable sector lost (%)
0	0
0.5	23
1.0	46
2.0	53
3.0	89
4.0	91

Photoreactivation exposures of plated cells lasted for thirty minutes at the indicated times. The non-photoreactivable sector after immediate photoreactivation was subtracted from the data, and the results expressed as the percent of the initial amount of photoreactivation that was lost.

Table 4

Loss of photoreactivation of the arg4-17 allele in excision defective strains after 2.0 Jm^{-2} UV radiation

post irradiation time (hours)	0	0.5	1.0	3.0	6.0
strain	photoreactivable sector lost (%)				
rad2-5	0	9	19	34	61
rad3-2	0	28	38	41	68
rad4-4	0	35	44	77	123
rad7-1	0	23	43	62	70
rad10-2	0	4	26	36	65
rad16-1	0	21	4	46	64

Photoreactivation exposures of plated cells lasted for thirty minutes at the indicated times. The non-photoreactivable sector after immediate photoreactivation was subtracted from the data, and the results expressed as the percent of the initial amount of photoreactivation that was lost.