For: DNA Repair and Mutagenesis in Eukaryotes
F. J. deSerres, W. M. Generoso and M. D. Shelby (Eds.), Plenum Press

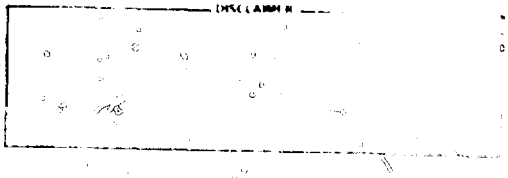
GENETIC AND PHYSIOLOGICAL FACTORS AFFECTING REPAIR AND MUTAGENESIS IN YEAST*

JEFFREY F. LEMONTT

Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

Running title: REPAIR AND MUTAGENESIS IN YEAST

Abbreviations: NA, nitrous acid; MMS, methyl methanesulfonate; EMS, ethyl methanesulfonate; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; HZ, hydrazine; YEPD, yeast extract - peptone-dextrose.



*Research sponsored by the Office of Health and Environmental Research, U. S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

Send proof to: Dr. J. F. Lemontt
Biology Division
Oak Ridge National Laboratory
Post Office Box Y
Oak Ridge, Tennessee 37830

Summary

Current views of DNA repair and mutagenesis in the yeast Saccharomyces cerevisiae are discussed in the light of recent data and with emphasis on the isolation and characterization of genetically well-defined mutations that affect DNA metabolism in general (including replication and recombination). Various "pathways" of repair are described, particularly in relation to their involvement in mutagenic mechanisms. In addition to genetic control, certain physiological factors such as "cell age," DNA replication, and the regulatory state of the mating-type locus are shown to also play a role in repair and mutagenesis.

Since the realization that DNA repair and mutagenesis in E. coli are closely related, enzymatically controlled cellular processes [8, 59, 174, 177], there has been a great deal of interest in determining whether repair mechanisms proposed for prokaryotes might also apply to eukaryotic cells. The axiomatic belief held by many that these more highly evolved cells would prove to be more complex in every way no doubt influenced many investigators to focus attention upon the unicellular uninucleate budding yeast Saccharomyces cerevisiae. This organism is among the simplest of all eukaryotes and offers several advantages over not only simpler prokaryotic systems but also more complex eukaryotic systems. These include: (1) ease of handling and low cost; (2) sophisticated genetic manipulation; (3) well-defined mitotic cell cycle in stable haploid, diploid, and polyploid strains; (4) relatively high tolerance of certain aneuploidies; (5) conjugation and meiotic development; (6) a nucleosome-dependent chromosomal organization; (7) a wealth of genetic and biochemical information concerning both nuclear-specific and mitochondrial-specific cellular processes [3, 5, 20, 51, 54, 118, 125, 151, 152, 156].

Attempts to explain the phenotypic traits of uvr, lex, rec, and other mutants of Escherichia coli, most notable of which is enhanced mutagen cytotoxicity, led inescapably to the hypothesis that this increased lethality is caused by the failure to repair DNA damage, provided that the genomes suffer the same number of initial lesions [68, 174]. Moreover, the proposal by Evelyn Witkin in 1967 [175] that different pathways of repair could represent either an accurate (error-proof or error-free) or an inaccurate (error-prone or mutagenic) molecular restoration of genetic information proved to be an important turning point in the way geneticists interpreted experiments

concerning repair effects on induced mutagenesis. Today, error-prone repair of UV damage in bacteria is belived to have an inducible component involving alteration or inhibition of the editing (nucleolytic) function of a DNA polymerase [78, 178].

In spite of efforts to extend the concept of error-prone repair to eukaryotic organisms, we still know very little about the nature of this repair, much less how the errors are made. Thus, it should be emphasized that without direct error frequency assays involving defined damaged-DNA substrates and purified repair enzymes, the error-prone repair concept represents only a descriptive albeit useful construct, just as it did in 1967. The evidence for error-prone repair depends upon the identification of one or more mutant genes conferring both increased mutagen sensitivity and defective induced mutability, compared with a wild type. Similarly, error-free repair in wild-type strains is inferred by virtue of its apparent absence in well-defined hypersensitive mutants that are also hypermutable (presumably because more lesions are repaired by error-prone repair). Obviously, these concepts cannot apply when chemical mutagens are subject to cellular metabolism unless numbers of DNA lesions are quantitated in both mutant and wild-type cells. This is one reason physical agents, particularly UV light and to a lesser extent ionizing radiations, have been so useful for quantitative studies of repair and mutagenesis.

There now exists a considerable body of information on repair and mutagenesis in Saccharomyces cerevisiae. Although certain aspects of this subject have been reviewed in the past [19, 56, 121, 166], the present summary attempts to broaden our current understanding not only by including more recent genetic data, but also by emphasizing the interrelation between

cellular processes that involve DNA metabolism, namely repair, mutagenesis, recombination, and replication; also included are developmental systems regulating the sexual cycle which affect chromatin indirectly.

Isolation of mutants with enhanced mutagen sensitivity

In 1967 Nakai and Matsumoto [122] were the first to describe radiosensitive mutants in yeast. They identified two separate mutant loci, now called rad1 and rad2 [38], that caused a significant loss of UV resistance; another, now called rad51 [37, 42], led primarily to X-ray sensitivity with only a slight effect on UV sensitivity. Snow [159] then reported 6 UV-sensitive mutants, each carrying different mutant loci, 4 of which were also hypersensitive to NA (but with a different rank order). Snow hypothesized that although repair of lesions inflicted by UV and NA would likely occur by non-identical enzymatic reactions, the different repair enzymes might represent components of one general repair system. Cox and Parry [22] then deliberately attempted to "saturate" the yeast genome with mutations conferring UV sensitivity in an effort to estimate the total number of independent genes responsible. Genetic analysis of 96 isolates revealed 22 separate mutant loci, 5 of which were also responsible for increased X-ray sensitivity. Subsequent isolations (Table 1) have revealed a large number of genetic loci controlling mutagen sensitivity, including 22 new genes detected in mutants selected for MMS sensitivity by Prakash and Prakash [137]. Of these, only 5 conferred MMS sensitivity alone, while others caused either UV (6) or X-ray (5) sensitivity as well, and 6 others led to cross-sensitivity to both radiations. T-1

Ananthaswamy et al. [1] recently attempted to "saturate" the yeast genome with mutations controlling X-ray sensitivity and found 15 new complementation groups, each complementing the existing rad set. Among such X-ray-sensitive mutants all but 4 were cross-sensitive to MMS, while none was cross-sensitive to UV. Although allelism tests to mms strains have not been performed, it is likely that several new unique genes are represented because the combined X-ray- and MMS-sensitivity phenotype of these 11 is expressed by only 5 of 22 mms loci.

To summarize thus far, UV-sensitive mutants comprise at least 19 genetic loci (rad1,2,3,4,7,10,13,14,16,19,20,21,22 and mms3,6,10,13,18,19); X-ray-sensitive mutants comprise 26-31 loci (rad50,51,52,53,54,55,56,57, xs3, xrs2,4, mms8,9,14,16,20, and an estimated 10-15 others); UV- and X-ray-sensitive mutants comprise 15 loci (rad5,6,8,9,11,12,15,17,18 and mms7, 11,12,15,17,21); and, finally, mutants sensitive to MMS but not to radiations comprise 5 loci (mms1,2,4,5,22). Since many but not all of the above 65-70 mutations have been shown to recombine with one another in meiosis, it is important to bear in mind the distinction between allelism and genetic complementation in estimating numbers of genetic loci. On the other hand, complementing repair-deficient mutants at the same chromosomal locus have not been reported.

Does this very large number of genes represent a reasonably accurate estimate for all possible mutants of this type? Although the answer to this question is not known, it does not seem likely to be a resounding "yes" for several reasons. On statistical grounds (based on the distribution of allelic repeats observed at 22 loci), Cox and Parry [22] estimated an additional 8-15 undetected mutable rad loci. With respect to the 22 new mms loci, Prakash and Prakash [137] have calculated a maximum likelihood

estimate of 48 ± 15 (S.D.) loci responsible for MMS sensitivity. Table 1 shows that mutants at some loci (e.g. rad1 or rad2) have been more readily detectable than others. Doubtless, this results from many factors, including the original strain employed (genetic background), the dose and type of mutagen used to enrich the mutant population prior to screening, the dose and type of mutagen used for screening hypersensitive strains, the conditions of treatment, and the level of effect arbitrarily chosen as a criterion for isolation. For example, the extreme UV sensitivity or rad1 and rad2 strains, a result of defective pyrimidine dimer excision repair [131, 164, 168], is probably a major reason such mutants have been repeatedly reisolated. Moreover, Prakash and Prakash [137] screened for the inability to grow in the continual presence of 0.5% MMS and found 3 loci (mms2,10,22) that did not confer MMS hypersensitivity when cells were exposed to brief MMS treatments in buffer. Thus, the permeability of MMS in such strains may be different under different conditions and in different genetic backgrounds. Since mms10 also enhances UV sensitivity, repair processes may be involved. Since mms2 and mms22 confer only enhanced MMS sensitivity (under certain conditions), MMS-specific repair processes may exist, or, alternatively, genetically altered MMS transport into the cell nucleus may be involved, again underscoring the need to compare strains having the same initial DNA damage.

Isolation of mutants affected in mutagenesis and recombination

Early recognition of the interrelation between DNA repair, mutagenesis, recombination, and replication in bacteria [68, 176] stimulated a number of yeast geneticists to ask whether these processes have anything in common

in eukaryotic organisms. In particular: (1) Are mutagenesis and recombination in yeast genetically controlled? (2) If so, how many genes are involved? (3) Do any of these genes function in repair-associated mechanisms? Table 2 shows that a number of genes have been identified in mutants selected for various genetic end points other than enhanced mutagen sensitivity. These include spontaneous and induced mutation as well as spontaneous and induced mitotic recombination. T-2

UV reversion-defective mutants

Using the vigorous UV-induced revertibility of the arg4-17 ochre allele to monitor induced mutability, Lemontt [93] screened clones (derived from cells surviving EMS treatment) for defective UV reversion. Upon genetic analysis 20 such isolates were found to comprise single recessive alleles of only 3 genes, called rev1, rev2 and rev3; rev2 was subsequently found to be allelic with UV-sensitive mutants isolated by Snow [159] and by Cox and Parry [22] and has since been renamed rad5 [38]. Mutations at any one of these 3 loci cause varying degrees of enhanced sensitivity to UV, X-rays, and EMS, implicating their involvement in some form of DNA repair. This suggests that UV mutagenesis in yeast is genetically controlled by an error-prone repair process, as had already been proposed for E. coli by Witkin [177].

The rev1 and rev3 genes were shown to cause large reductions in UV mutation frequencies compared with the wild type, not only for reversion of arg4-17 (ochre), lys1-1 (ochre), and arg4-6 (putative missense) [93], but also for forward mutation at biosynthetic loci across the genome leading to auxotrophic requirements and for forward mutation at 2 specific ADE loci

(ade1 or ade2) [150] causing red-pigmented clones [95]. Conversely rev2 had much smaller effects at arg4-17 and lys1-1 and at biosynthetic loci yet had no significant effect at all on UV reversion of arg4-6 or on forward mutation at the ADE loci [93, 95]. Moreover, the average effect of rev1 across the genome (4% of the wild-type response) was much greater than at the 2 selected ade genes (19% of wild type), whereas the effect of rev3 was large in both cases (4 and 2% of wild type, respectively) [95]. This was one of the first indications that UV mutagenesis might not be acting uniformly at all genetic sites, i.e. a hot-spotting effect or a specificity of interaction between certain mutagenic (error-prone repair) enzymes and particular genomic regions or particular types of DNA damage. It was also suggested that the rev2 block is highly specific, perhaps affecting only UV reversion of ochre alleles.

More recently, extensive data of Lawrence and Christensen concerning the effect of rev genes on UV rerversions of well-defined cyc1 (iso-1-cytochrome c) alleles has for the most part confiremed these earlier suggestions of specificity and non-randomness of UV mutagenesis [88, 89, 91]. In addition, they have identified several other mutant rad loci that reduce UV mutagenesis [87] — rad6, rad8, rad9, and rad18. These all cause enhanced sensitivity to both UV and X-rays, like the rev genes and like recA and lex genes of E. coli [177]. Unlike lexA, however, which is dominant over the wild-type allele [119], rad and rev genes involved in UV mutagenesis are all recessive in their effects on survival and induced mutation, suggesting the loss of required enzymatic steps in the mutagenic mechanism.

Forward mutation at the CAN1 locus

Unlike reversion, recessive forward mutation usually results in the loss of an essential cellular function and, in principle, can derive from various mutational alterations. Many systems used to quantitate forward mutation (e.g. auxotrophy, pigmented clones, lethals) have limited utility mainly because they are non-selective and therefore relatively inconvenient. Many systems measure mutation at any one of a large number of genetic loci. Forward mutation at CAN1, however, represents a convenient, selective drug-resistance system that is sensitive to many physical and chemical mutagens [11, 45, 49, 83, 84, 99, 100, 141, 170].

Recessive can1 mutants become resistant to the highly toxic arginine analogue, canavanine, by mutational alteration of CAN1 on chromosome V, believed to be the structural gene for the arginine-specific permease enzyme [49, 170]. Since this permease transports virtually all exogenous arginine (and canavanine) into the cell under normal conditions of ammonia repression when general amino-acid permeases are inactive, all such canavanine-resistant mutants map at this one genetic locus [47—49]. Intragenic (interallelic) complementation has not been observed even among a large number of unique dihybrids, suggesting that arginine permease is functional as a single polypeptide [170]. Fine-structure mapping of alleles yielding the greatest recombination is suggestive of enough DNA to code for a protein as large as 260,000 daltons [170]. The molecular weight of arginine permease is not known, but it could be considerably less if (1) the correspondence between gene and protein for this mapping method [81, 110, 124] is unreliable for CAN1 (the largest gene presumed to exist

in yeast), as appears to be the case for very small genome intervals [115, 116], or if (2) certain portions of the gene are non-structural and are subject to post-transcriptional or post-translational processing critical for functional integration of the permease into the cell membrane. Thus, mutations in non-coding but critical sequences could also result in inactive permease; and unlike most mutable genes used in mutation studies, which usually affect soluble enzyme activities, CAN1 is responsible for the activity of an important membrane protein that must be synthesized (presumably on cytoplasmic ribosomes) and subseqeutnly transported and integrated into the cell envelope in some specific way.

In wild-type yeast grown to stationary phase in a YEPD complex broth, it is observed that many mutagens, including UV, cause vigorous induction of can1 mutants and that these are readily expressed on selective agar containing the drug, presumably before canavanine toxicity becomes too great [45, 99-101]. These findings are believed to be due in part to a relatively high turnover rate of the permease such that mutational expression is strongly influenced by the cellular level of endogenous free arginine (dependent upon type of growth medium) rather than by the ability to undergo residual divisions on the plate [45]. This is consistent with the general observation that canavanine cytotoxicity is dependent upon the exogenous ratio of canavanine to arginine such that defective canavanyl proteins are eventually synthesized. Thus, for pre-growth in YEPD broth media the free arginine pool is presumably high enough to prevent significant toxicity during a period when the permease activity is decaying rapidly.

UV forward mutation-defective mutants

In an effort to identify new genes controlling UV mutagenesis or its expression at CAN1, Lemontt screened for clones (YEPD pre-growth) with less than wild-type levels of UV mutation to canavanine resistance [96]. Such ultraviolet mutation-resistant isolates were subsequently characterized and found to carry one of 7 non-linked recessive umr alleles [100]. The umr loci did not cause canavanine resistance and were not linked to can1, nor could they be explained by an extra (disomic) copy of chromosome V. Unlike rev or rad mutants, 4 of these genes (umr4, umr5, umr6, umr7) had no significant effect on either the UV sensitivity or the UV revertibility of 3 ochre mutations, his5-2, lys1-1, and ura4-1. Diploids homozygous for umr5, umr6, or umr7 all failed to sporulate, suggesting a meiotic defect [100]. The umr7 locus, known to be allelic with and mapping in the same region as tup1 and cyc9 on chromosome III, has an exceedingly rich pleiotropic phenotype with effects on conjugation (α-specific poor mating ability), the cell surface (extreme flocculence or cell clumping and "self-shmooing"), and membrane-associated functions (dTMP uptake and unusually high levels of iso-2-cytochrome c) [100, 104, 105, 153, 171]. The UMR4, UMR5, UMR6 and UMR7 genes may be more concerned with expression of can1 mutations rather than with mechanisms of mutagenesis directly [101]. On the other hand, umr1, umr2 and umr3 mutants were slightly more UV-sensitive than the wild type and were affected to varying degrees in UV revertibility of one or another of the 3 ochre alleles [100]. This is consistent with the idea that one or more of these latter 3 UMR genes are concerned with highly specific branches of mutagenic pathways, those contributing very little to the overall repair potential of the cell. Homozygotes of umr2 and umr3

failed to sporulate. However, since all umr loci except umr1 also led to increased canavanine toxicity, and since UV-induced can1 mutation frequencies may be boosted if selection is delayed and preceded by a period of cell division in growth medium [101], it is possible that there has been a genetic alteration either in the arginine pool size or in the rate or quality of arginine permease turnover processes. Thus, UMR1 seems most likely to be involved in mutagenic repair pathways; this is further supported by the finding that umr1 rad2 and umr1 rad6 double-mutant haploids are much more UV-sensitive (synergistic) than the respective single rad strains [103]. On the other hand, the involvement of UMR2 and UMR3 in mutagenic pathways remains more tentative.

Hypo-rec, hyper-rec, and mutator strains

In 1964 Holliday [62] proposed a molecular model for gene conversion in fungi implicating a role for repair enzymes such as nucleases, polymerases, and ligases. The model involves breakage and reannealing of complementary DNA strands of homologous chromatids to generate a "hybrid" region. If the region includes a heterozygous mutation, this hybrid DNA will contain one or more mispaired bases (mismatched or heteroduplex DNA), a substrate for repair. Recombination-deficient mutants of E. coli were found to be radiation sensitive [18]. Radiation-sensitive mutants of Ustilago maydis [63] were recombination-deficient [64]. Further, post-replication repair of daughter-strand gaps in DNA [154] appeared to require recA$^+$-dependent recombination ability [158]. All these findings had the effect of intensifying the search for genes affecting recombination in yeast. It should be emphasized that recessive rec mutants are not easily selected

by conventional genetic means since single mutational events in one chromosome are not expressed in heterozygous diploids. Instead, radiation-sensitive mutants were routinely examined (in homozygous condition) for effects on either meiotic or mitotic recombination. Early indications were that many rad genes had no effect in meiosis, whereas rad/rad mitotic cells generally expressed higher levels of radiation-induced recombination than comparable RAD/RAD or RAD/rad diploids at equal exposures, suggesting that unrepaired radiation damage to DNA is recombinogenic [94, 160].

In a more direct approach Rodarte-Ramon and Mortimer [148, 149] selected rec mutants directly on the basis of defective X-ray-induced mitotic gene conversion at arg4. They constructed a strain disomic (n+1) and heteroallelic for arg4 on chromosome VIII, thereby permitting expression of rec genes on any of the other haploid chromosomes. Seven genes were identified, two of which conferred X-ray sensitivity, one of which caused UV and X-ray sensitivity, and four of which had no effect on radiation survival; rec2 (X-ray sensitive) was later found to be allelic with rad52 [43]. It was suggested that enzymatic steps required for induced recombination in yeast might also be shared by certain repair pathways.

With a similar system Maloney and Fogel [108] screened heteroallelic arg4 disomics for enhanced spontaneous mitotic gene conversion. Several genes have been identified and are believed to be affected in a regulatory mechanism that normally keeps mitotic recombination at a low level. (There exists genetic evidence for such repression of mitotic intragenic recombination [33]). Some of these mutants exhibit enhanced sensitivity to MMS, UV, or X-rays, implicating a role of DNA repair in the regulation of

spontaneous mitotic recombination. This is supported by the results of Prakash and Prakash [138] who found that homozygotes of mms8, mms9, mms13, or mms21 exhibit a hyper-rec phenotype (increased spontaneous mitotic segregation from CAN1/can1 to can1/can1); rad18 also shows the hyper-rec phenotype [7].

As summarized in a review by Resnick [144], it should be emphasized that mitotic recombination of alleles within a gene is observed to be predominantly a non-reciprocal process (i.e. gene conversion) rather than a result of reciprocal crossing-over events, whereas the reverse is generally true of intergenic recombination, particularly after the frequency has been raised by exposure to external agents [123]; and, most mutagens are good recombinogens. In either case, mutational alteration of one homolog occurs much too infrequently to account for mitotic recombination in yeast and other fungi. The implications for mammalian somatic cell mutagenesis should be clear: The induction of autosomal recessive "mutants" in cell lines believed to be already heterozygous [16] may in fact be induction of mitotic crossing-over anywhere between the genetic locus and its centromere, or, to a lesser extent, gene conversion (assuming that chromosome loss, deletion, and non-disjunction can be excluded).

Probing the potential relation between DNA repair and spontaneous mutability, von Borstel and his co-workers found that the spontaneous mutation rate is increased by several rad genes — rad18, rad52, xs3 [165], and, more recently, rad3, rad6, rad51 [55]. In direct screening for such mutators [167], a minimum of 8 genetic loci have been identified [55]; one, MUT6, is dominant and without effect on UV, X-ray, or MMS sensitivity; among the other 7 recessive mut loci, all but one (mut1) sensitize cells to one or more of

these mutagens; mut5 has been reported to be allelic with rad51. These authors believe that spontaneous DNA lesions (including replication errors) are susceptible to repair by systems having several steps in common with systems that repair mutagen-induced DNA damage. The isolation of antimutator strains has also been reported [140]. In addition, rev3 has antimutator activity [139].

Previous work with bacteriophage T4 mutator [161] and antimutator [28] strains has suggested that the exonucleolytic activity associated with the polymerase has a proofreading or editing function that normally corrects (repairs) spontaneous replicative errors, presumably mismatched bases [58, 155]. The inducible component of UV-induced mutagenesis in E. coli also appears to be associated with some process that permits the replicase (DNA polymerase III) to make errors at higher than normal frequency [9]. Whether yeast and higher eukaryotic cells have the same or a similar mechanism is not known. It does seem clear, however, that spontaneous mutability is genetically controlled in a complex way that is not entirely independent of repair mechanisms.

There is evidence that spontaneous mutagenesis and mitotic recombination are under joint genetic control in yeast. Using a procedure to select dominant or recessive mutations affecting spontaneous forward mutability at CAN1, Golin and Esposito [46] have described a semi-dominant mutation, reml-1, that elevates the spontaneous rates of both mutagenesis and mitotic recombination. Meiotic recombination is not affected by this mutation even when homozygous, but ascopore viability is reduced, suggesting a meiotic defect in chromosomal integrity or disjunction. These authors feel that

spontaneous mutation and recombination are enhanced as a result of an increase in specific DNA structures such as mismatched base pairs or single-stranded regions. A previously selected meiotic mutation, spo7-1, isolated as sporulation deficient has been shown to be responsible for both antimutator activity (mitotic) and defective pre-meiotic DNA synthesis [31]. There is now a good correlation between meiotic deficiencies and certain X-ray-sensitive rad mutants [42, 44]; sporulative ability is reduced in homozygotes of rad51 and rad55; rad50, rad52, and rad57 homozygotes do sporulate, but nearly all meiotic products are inviable, analogous to mei mutants of Drosophila [4] and rec mutants of Ustilago [66, 67]. Sporulation is completely abolished by rad6-1. Recent results by Game et al. [44] show that RAD50, RAD52, and RAD57 are not required for early and late meiotic events (namely pre-meiotic DNA synthesis and sporulation, respectively) but are required for successful meiotic recombination.

To summarize, there are now a large number of genes in yeast believed to control various aspects of DNA repair, mutagenesis, and recombination. Mutants selected on the basis of one altered property often turn out to be pleiotropic with respect to another phenotypic trait. To this extent certain yeast mutants appear analogous to bacterial mutants affected in some aspect of DNA metabolism. Clearly, not all of these genes may be concerned with DNA repair directly, as discussed by previous authors [22, 56, 137]. The challenge to define in molecular terms cellular functions gone awry in nearly 100 (and potentially more) mutants underscores and provides evidence for the enormous complexity of eukaryotic DNA-related metabolism. The existence of dominant and semi-dominant mutations that jointly affect repair,

mutagenesis, and recombination in yeast raises the possibility that induction of such mutations in somatic tissues of mammals, for example, might also serve to increase (by recombination) the overall rate of homozygosis of deleterious heterozygous recessive loci. In this way, induction of hyper-rec mutants might increase the cancer risk.

"Pathways" of Repair

It has been possible to characterize presumed repair-deficient mutations by their interactions in multiply-mutant haploid strains, as developed by Game and Cox [39, 40] and by Haynes [56]. Howard-Flanders et al. [69] were among the first to demonstrate the utility of this approach for understanding DNA repair mechanisms by constructing a double mutant of E. coli carrying both uvrA and recA and showing that the two mutations interacted synergistically with regard to UV sensitivity. That is, UV survival of this double mutant was very much less than what would have been expected on the basis of an additive effect of the two single mutants; further the UV dose yielding an average of one lethal event (37% survival) corresponded to approximately one pyrimidine dimer per cell. This result suggested two important hypotheses: (1) that uvrA and recA each block very different repair pathways acting on UV-damaged DNA; (2) that these two major pathways could account for virtually all of the UV resistance exhibited by the wild type. This agreed with the finding that uvrA mutants lacked excision repair but recA mutants did not [68]. Two or more mutations blocking DNA repair along the same linear pathway are expected to interact epistatically such that the multiple mutant is no more sensitive to the mutagen than the most sensitive single mutant.

Despite some inherent limitations of multiple mutant analysis, as discussed in detail elsewhere [39, 40, 87], it has been possible to gain a certain amount of information concerning mechanisms of repair in yeast from this kind of approach in conjunction with other phenotypic traits expressed by repair-deficient mutants. On this basis, there exists 3 so-called "epistasis groups" of rad loci such that a strain carrying multiple mutations within a group exhibits epistasis whereas a strain with mutations in different groups exhibits either an additive or synergistic interaction. The epistasis groups are suggestive of metabolic pathways [21].

Excision repair of UV damage

The epistasis group defined by rad1, rad2, rad3, rad4, rad10, and rad16 consists of mutants with a biochemical defect in excision repair of UV-induced pyrimidine dimers [39, 131, 134, 135, 146, 147, 164, 168]; furthermore, rad22 is epistatic to rad1 with respect to UV survival [87]. Thus, at least 7 genes appear to be required for excision repair in yeast. Excision repair acts only on nuclear DNA and is not able to remove pyrimidine dimers from mitochondrial DNA [131, 169]. In general, mutations in this pathway do not lead to X-ray sensitivity, nor do they have any effect on recombination, meiosis, or sporulation. Additionally, like uvr mutants of E. coli [177], most if not all of these mutants exhibit enhanced frequencies of UV mutagenesis compared with the wild type at equal UV doses, and a significant fraction of the induced mutability is photoreversible in both mutant and wild-type strains [2, 87, 92, 143, 179]. At equal survival levels induced mutabilities are approximately the same in RAD and rad2 strains [30]. Moreover, with respect to the observed spectrum of base-pair changes inferred from amino-acid replacements in iso-1-cytochrome c

among UV revertants of ochre cyc1 alleles [92], and rad1 response is the same as that produced by the wild type. All these findings have suggested that UV mutations are produced predominantly from unexcised pyrimidine dimers in DNA by a mutagenic process different from the excision repair pathway which is considered to be essentially error-free.

Error-prone repair of UV damage

The epistasis group defined by rad6, rad8, rad9, rad18, rev1, rev2 (i.e. rad5), and rev3 consists of mutants with varying degrees of both UV and X-ray sensitivity [21, 22, 40, 87, 93]. None except rad9 significantly reduces mitotic recombination [82]; rad6 prevents sporulation [22]; and the others do not apparently affect meiosis. The rad18 gene is synergistic with rad1, rad2, or rad3 but epistatic with rad6 [40]. All 7 mutants of this group are epistatic with rad6, suggesting that all are involved in one major pathway concerned with repair of UV damage [87]. Mutants carrying rad6 or rad9 are proficient in carrying out pyrimidine dimer excision [135]; the others are also excision-proficient (see ref. [19]).

The most interesting property of mutants in this group is defective UV mutagenesis, suggesting, by analogy to recA and lexA mutants of E. coli [87, 96], that this single repair pathway is error-prone for UV damage. From this point on, the analogy to prokaryotic mechanisms of mutagenesis begins to break down. Lawrence and Christensen [88, 90] have pointed out how many of the observations in yeast are at best difficult to explain with the one-step unitary model proposed for E. coli [12, 178], according to which suppression of the editing function of DNA polymerase permits replication past a pyrimidine dimer while two random, often incorrect

bases are inserted opposite the lesion. Thus, recA and lexA mutations should prevent induction of mutations of all types and at all genetic sites, and mutations induced in the wild type should involve double base-pair changes [88, 90].

Lawrence et al. [92] have demonstrated that rad6 and rad18 affect UV mutagenesis not only quantitatively but also qualitatively by altering the spectrum of base changes observed among induced revertants of cyc1 nonsense alleles. Not all mutants defining error-prone repair in yeast block UV-induced mutational changes of all types and at all genetic sites; and, double base-pair changes are rare in yeast [90]. While rev3 and rad6 are non-specific and prevent normal levels of UV mutagenesis at every genetic site tested, the remaining mutants of this pathway have strong allele-specific effects with respect to UV reversion (for examples see Table 3). T-3

The REV2 gene product appears to be concerned only with UV reversion of ochre alleles, yet, this clearly is not the case for all such alleles. Although the REV1 product may not be required for frameshift mutagenesis by UV, it is required for many but not all base-pair transitions and transversions. By inspection of the nearly complete base sequence information in the region of many cyc1 alleles and of their revertants, it has been possible to test the idea that the allele specificity of UV reversion (as typified by rev1) may be based upon one or more of the following factors: (1) position within the gene, (2) kind of DNA triplet altered, (3) type of base-pair change (e.g. transition vs transversion, or AT to GC vs GC to AT), (4) variable recovery of reversions (5) non-random formation of dimers in regions rich in adjacent pyrimidines,(6) unusual kinds of premutational

lesions, or (7) different ratios of mutagenic to non-mutagenic repair at different genetic sites. Lawrence and Christensen have concluded that none of these factors alone can satisfactorialy account for the non-randomness of UV reversion [88, 90]. Even in a wild-type strain the same ochre triplet occuring at different sites reverts with entirely different patterns of base-pair change, suggestive of some form of site (or sequence) specificity [157]. Moreover, UV reversion of the cyc1-131 allele by GC to AT transition, which does not require REV1 but which is nonetheless photoreversible, occurs within an alternating purine-pyrimidine nucleotide sequence, obviously a region where intrastrand pyrimidine dimers cannot be induced [88, 90]. Thus, we also need to understand how DNA damage at one site results in mutation at another.

Thus far, the best explanation for site-specific mutagenesis is a presumed non-random interaction between certain gene products (of error-prone repair) and DNA damage in particular genomic regions [90]. It is not known whether this surprising level of complexity is unique to yeast or to eukaryotes in general. Bacterial studies in the past have for the most part not been concerned with this question of specificity. Not until very recently have mutation-resistant ("rev-like") mutants of E. coli been selected directly [74]. If such apparent site-specific regulation of mutagenesis is found to be unique to eukaryotes, the molecular environment of the chromatin is likely to play a role.

Minor repair pathway for UV damage

A third "minor pathway" for repair of UV damage appears to involve enzymatic steps whose major ostensible function is to repair ionizing radiation-damaged DNA. The rad50 and rad51 genes each confer slight UV sensitivity and are epistatic to one another, yet rad51 interacts synergistically with both rad3 and rad18 with respect to UV survival. One lethal event (37% survival) in rad3 rad18 double mutants corresponds to approximately 6 pyrimidine dimers per cell, whereas in rad3 rad18 rad51 triple mutants only 1 or 2 are needed to produce the same effect. This suggests that unrepaired dimers are lethal and that virtually all of the UV resistance expressed by the wild type can be accounted for by the action of these 3 pathways [21,40]. The rad52 locus also acts in this minor pathway, but has little or no effect on UV reversion. In excision-defective strains, however, a rad52 rad1 strain is nearly 10-fold more UV hypermutable than a rad1 strain, suggesting that this minor pathway is essentially error-free for repair of UV damage [87].

Repair of ionizing radiation damage

The major pathway for repair of ionizing radiation damage is controlled by RAD50, RAD51, . . ., RAD57; rad52 contributes the greatest gamma-ray hypersensitivity, which is also exhibited by all double mutants with rad52 [113]. Frequencies of gamma-ray reversion in mutants of this group are similar to that expressed by the wild type, suggesting an error-free mode of repair [112]. Since rad52 strains are defective in gamma-ray-induced mitotic gene conversion [148, 149] and are also unable to repair double-strand DNA breaks [60, 145], this RAD52 pathway may involve recombinational

repair; rad52 strains also have increased X-ray-induced dominant lethality, suggestive of a defect in the repair of chromosome breaks [61].

With respect to ionizing radiation survival and mutagenesis, McKee and Lawrence [112, 113] have found that the single mutagenic repair system for UV is also responsible for mutagenic repair of ionizing radiation damage and requires the functions of the RAD6, RAD8, REV1, REV2, and REV3 genes. Although both radiations produce very different kinds of pre-mutational DNA damage, gamma-ray mutagenesis is efficiently blocked by mutations of these loci, all of which comprise a "rad6 epistasis group" for gamma-ray survival; rad9 and rad18 also belongs to this group but do not block gamma-ray mutagenesis significantly, suggesting that this RAD6 pathway consists of both error-free and error-prone repair processes. In addition, McKee and Lawrence [114] have observed in rev strains allele-specific gamma-ray reversion patterns that are very similar to those expressed after UV exposure. These authors have argued that the simple idea of an enzymatic pathway for mutagenic repair consisting of sequential gene-controlled steps, with separate branch points leading to mutational specificity, does not adequately explain the distinctive yet partially overlapping mutational phenotypes expressed by mutants of this "pathway".

Mutagenesis by several chemical agents also requires a functional repair system, specifically the RAD6 and RAD9 gene products [130, 132, 133]. McKee and Lawrence [114] argue that mutations of different kinds or at different sites that arise from potentially very different premutational lesions are produced by the coordinate action of a large number of partially independent sets of gene functions.

Physiological factors affecting repair and mutagenesis

Early studies on the recovery of yeast from radiation and chemical damage showed that the degree of liquid-holding recovery, an indicator of repair activity, could be modified by different physiological conditions [128, 129]. More recent studies [127] not only have emphasized the importance of genetically controlled repair processes but also have expanded our view of the diversity of cellular factors that can affect repair. These include "cell age," DNA replication, and the mating-type-dependent regulatory system.

Cell age

The term cell age, as developed by Parry and co-workers [126], encompasses two different phenomena -- either the position in the mitotic cell cycle of synchronous cultures or the transition of exponentially growing (log-phase) asynchronous cultures to a nutrient-limited stationary phase. The increased radiation resistance of the budding cell fraction of yeast cultures observed in early studies [6] is now understood to be a reflection of the hypersensitive G_1 and early S (DNA replication) stages, compared with the more resistant late S and G_2 periods [15, 26, 27]. In general, this pattern is similar to that first observed in mammalian cells [163]. The variations in UV resistance could be due to different amounts of initial DNA damage induced at different times in the cell cycle or to varying efficiencies of repair mechanisms throughout the cycle. Although S-phase cells suffer 30% fewer pyrimidine dimers per unit UV dose than do cells having minimal resistance [14], this factor is not likely to be

responsible for the bulk of the observed variations in UV sensitivity [25]. An excision repair-deficient (rad1) strain exhibits cyclic variations in UV sensitivity very similar to those found in wild type, suggesting that excision repair acts efficiently and uniformly throughout the cell cycle [13]. In contrast a rec5 strain, defective in UV-induced mitotic recombination, exhibits the same UV sensitivity in G_1 and G_2, a level comparable to the wild-type G_1 level. This suggests that the increased G_2 (over G_1) UV resistance expressed by the wild type is due to a recombinational repair process that requires the REC5 gene product [13].

Davis et al. [25] used a zonal rotor centrifugation method to isolate large yeast populations on the basis of bud size (correlated with progress in the cell cycle), an obvious improvement over the use of perturbing treatments that induce cell synchronony. Their results confirm earlier observations that, in the wild type, UV resistance is minimal in G_1 and maximum in S and G_2; yields of UV-induced mitotic recombination (intergenic and intragenic) were maximum in G_1 and minimum in S and G_2, again suggesting a relationship between cell survival and recombination. Fabre [32] has shown that UV-induced intragenic mitotic recombination can occur in G_1 before chromosome replication, confirming earlier results [172] and suggesting that homologous chromosomal pairing does not require duplicated chromatids. In contrast to UV survival, nitrous acid survival exhibited a minimum during only one period, that of DNA replication, while induced mitotic recombination occurred at all stages but was maximum during S [25].

Radiation and chemical mutagen sensitivity have also been compared in log-phase vs stationary-phase cultures [126]. As asynchronous log-phase cultures enter a transition period before entering stationary phase, cells

tend to complete their cycles and begin arresting as unbudded cells (in G_1 or more properly G_0) [54]. Stationary-phase cells are observed to be more sensitive to UV and X-rays than log-phase cells, whereas just the reverse is true of sensitivity to several chemical mutagens. During the transition period, UV resistance begins to decrease in cultures that have already begun to show a significant reduction in the frequency of budded cells. While excision repair-defective (rad1 or rad2) strains also become more UV sensitive, a rad50 strain failed to exhibit this effect, suggesting a requirement for the RAD50 gene product. In contrast to UV survival, cell survival following treatment with NA, mitomycin C, and EMS increases in cultures beginning to show loss of budded cells. A significant fraction of this differential chemical mutagen sensitivity appears to be due to different numbers of initial DNA lesions inflicted, since cellular uptake of tritium-labeled EMS is 7-fold less in stationary cells compared with log-phase cells [126].

DNA replication

Until recently very little information has been available on the role of DNA replication in repair and mutagenesis of yeast. The main reason for this has been that only a few temperature-sensitive mutants have been described [52, 53, 72] that have large effects in turning off DNA synthesis specifically and rapidly after temperature shift. Some mutants also affect RNA synthesis; the gene products of many well-defined mutations are not known. It has been suggested that a "replication complex" with one defective protein component might undergo slight conformational changes and still have some polymerizing activity [72]. Another reason concerns

the fact that most in vivo studies eventually depend upon the conventional end point of colony (or mutant colony) formation, which in turn is dependent upon genome replication and cell division.

Yeast strains carrying cdc8 are defective in DNA replication (elongation, not initiation) at 36°C but not at 23°C [52]. Prakash et al. [136] have reported recently that cdc8 reduces frequencies of UV reversion (at 25°C), even in rad1 or rad51 strains; they argue that CDC8 plays a role in error-prone repair.

There now exists evidence that the temperature-sensitive cdc9-1 mutant [24] is defective in DNA ligase activity [73]. At the restrictive temperature this mutant (1) accumulates many single-strand breaks in DNA, (2) exhibits enhanced UV sensitivity, and (3) produces enhanced frequencies of spontaneous mitotic recombination (hyper-rec phenotype) [41]. These recent findings underscore the multiple role of this enzyme in DNA replication, repair, and recombination in yeast. It is suggested [41] that the excess single-strand gaps in DNA are themselves recombinogenic, either directly or by means of the induction of a recombination-repair system.

An important question concerns the kinetics of induced mutagenesis: Does it occur before, during, or after DNA replication? One approach might involve the use of a probe that can monitor the appearance of mutant (or recombinant) gene product as soon as it becomes expressed, rather than the phenotypic scoring of mutant (or recombinant) clones many generations removed from the initial mutagenic (or recombinogenic) event. Such a system has been previously used in Ustilago maydis to allow in vivo enzymatic measurement of radiation-induced mutation [97, 98] or mitotic

recombination [65]. Another approach involves unambiguous detection of the "strandedness" of induced mutations. That is, fixation of pre-mutational damage in one strand of unreplicated (G_1-phase) DNA is expected to give rise to a mosaic colony because after completion of the first cell cycle there will be one mutant and one non-mutant cell. Damage fixed as mutation in both strands prior to replication should lead to a pure mutant clone. Pre-mutational damage not fixed as mutation until after the first round of replication will also produce mosaic clones.

James and Kilbey [70] observed UV induction of recessive lethal mutations in mitotic pedigrees of irradiated G_1 diploid yeast cells. With this technique they found that after low exposures to UV, induced mutations were produced in an excision repair-proficient strain prior to the first round of post-irradiation DNA replication, and most mutations were 2-stranded. In an excision repair-defective (radl) strain, induced mutations affecting both strands were not observed; moreover, mosaics arose as frequently in the second post-UV generation as in the first [71]. In radl strains unexcised pyrimidine dimers were shown to be responsible for UV mutagenesis even after passing through several DNA replication cycles [76], as Bridges and Munson had shown many years ago for E. coli [10]. Hannan et al. [50] had previously shown that G_1 RAD haploid cells produced exclusively pure mutant clones after a UV exposure leading to high survival (63%, on the survival curve "shoulder"). Mosaics, however, were produced with increasing frequency for UV doses corresponding to exponentially decreasing survival, and they could not be explained by first-division lethal sectoring. These

findings support the idea that pure mutant clones are associated with efficient heteroduplex repair activity such that loss of efficiency at higher UV doses leads to the induction of mosaics.

Kilbey et al. [75] have proposed a dimer/gap model to account for the different kinetics of UV mutagenesis in RAD and radl strains. According to the model, mutation fixation by error-prone repair is presumed to be initiated in both strains by a structure consisting of a single-stranded gap opposite a pyrimidine dimer, although this structure is produced in different ways by the two strains. In RAD strains after UV exposure sufficient to induce dimers close together on opposite complementary strands, the excision of one may often leave a gap that exposes the other. Since pre-replicative mutagenesis (presumably an error-prone gap-filling process) eventually affects both DNA strands [70], excision repair must remove the dimer or heteroduplex repair must recognize and repair the mismatched site. In radl strains excision cannot occur, and replication presumably generates daughter-strand gaps opposite pyrimidine dimers, followed by gap-filling and heteroduplex repair. This model is consistent with the observation that UV mutagenesis in RAD strains exhibits dose-squared dependence (2 dimers required) [75, 89, 100] compared with a linear dependence at low doses in radl [75] or rad2 [29] strains. Yet, there exists at least one case of linear induction in RAD strain [30]. It is not altogether clear just how gap-filling generates single base-pair changes, which are responsible for the majority of UV mutations in yeast [90] rather than double base substitutions, as presumed in the bacterial model [12, 178]. An error-prone gap-filling model must also accommodate in some way the observations of non-random action (site specificity of repair) and "mutation at a distance" [90].

Recent studies by Lemontt [99] have suggested that exposure of YEPD-grown stationary-phase yeast to HZ (a carcinogen and mutagen in other organisms [77]) results in pre-mutational DNA damage that becomes fixed as mutation at the time of DNA replication, as appears to be the case in Haemophilus [79, 80]. Unlike several other mutagens, HZ mutability at CAN1 is entirely dependent upon post-treatment DNA replication and occurs over a dose range that leaves cell viability unaffected. HZ exposure does not extend the 3- to 4-h growth lag normally observed in post-treatment medium. Prokaryotic studies have suggested that N^4-aminocytosine may represent an important pre-mutational DNA lesion to HZ-exposed cells [77]. Thus, unlike non-pairable pyrimidine dimers, this cytosine analogue might be considered a pairable lesion (with perhaps less than complete fidelity) produced in situ. N^4-aminocytosine is known to be mutagenic in lambda phage and in E. coli when used as a precursor for DNA replication [17]. Thus, HZ may induce mutations in yeast by a mechanism of base mispairing at replication in the absence of any ostensible inhibitory effect on the replicative process itself, as proposed for Haemophilus. Kimball has suggested that N^4-aminocytosine may be an intrinsically more efficient base-analogue mutagen than 5-bromouracil because the hydrazino ($-NH_2NH_2$) substitution for the 4-amino group occurs at a base-pairing position on the pyrimidine ring, while the bromine substitution does not [78].

It has also been possible to obtain indirect evidence for pre-replicative error-free repair of HZ-induced pre-mutational damage [102]. As observed in Haemophilus [80], if post-treatment DNA replication or its initiation is delayed in growth medium (in yeast with hydroxyurea or cycloheximide,

respectively), the maximum level of replication-dependent mutagenesis attainable after removal of inhibitors decreases (Table 4). Excision repair-defective rad2-1 strains also exhibit such loss of HZ mutability. These findings have suggested that pre-mutational lesions are being removed by some error-free process different from excision repair of pyrimidine dimers. Since cycloheximide blocks protein synthesis (which is required for initiation of DNA replication in yeast [57, 173]), this repair process must be constitutive. It is possible to speculate that some form of mismatch repair may be operating. If it is assumed that N^4-aminocytosine is a major pre-mutational lesion, the duplex distortion (presumed to occur by virtue of a hydrazino rather than an amino proton donor in hydrogen bonding to guanine) might also be correctable by specific cleavage of the terminal amino group, restoring normal base pairing without the need for strand breaks in the backbone. Examining HZ-treated Haemophilus, Kimball and Hirsch [80] failed to detect single-strand breaks (or alkali-labile sites) in unreplicated DNA, nor did they observe gaps in newly synthesized DNA.

T-4

Mating-type locus-dependent regulation

Normal conjugation in yeast occurs between cells of opposite mating type, either a or α. These two mating phenotypes segregate in meiosis as different alleles of the same locus, called the mating-type locus (MAT). There are now many lines of evidence supporting the idea that the genetic information at MAT has a regulatory function that plays a central role in controlling whether a cell may undergo sexual conjugation or pursue meiotic development. Diploids exhibit one of three possible functional states at MAT -- a/a, α/α, or a/α. Like a or α haploids, homozygous a/a or α/α diploids (selected by

mitotic crossing-over) are able to mate normally (and produce and respond to mating pheromones), exhibit medial bud initiation in mitosis, but cannot initiate meiosis and sporulation when challenged to do so in the appropriate medium. Such homozygotes exhibit a-specific or α-specific functions expressed by haploids. Diploids heterozygous (a/α) at MAT are repressed in mating ability (and fail to produce or respond to mating pheromones), exhibit polar bud initiation, and have gained meiotic and sporulative capacity. The a/α state appears not only to turn off certain haploid functions, but also to turn on certain new diploid functions. Moreover, the expression of these a-specific, α-specific, and a/α-specific functions may be altered by various mutations in several genes unlinked to MAT (for recent reviews of mating-type-specific functions, see refs. [23, 107, 109]). Thus, in mitotic cells the existence of phenotypic differences expressed by MAT homozygotes compared with the "normal" (after normal a x α mating) MAT heterozygotes constitutes evidence for MAT regulation.

DNA repair, mitotic recombination, and mutagenesis all appear to be modulated to some degree by MAT. Although diploids are much more X-ray-resistant than haploids (the ploidy effect [117]), MAT homozygotes are more sensitive than a/α cells [85, 117], suggesting that a fraction of the extra diploid resistance is due to MAT heterozygosity. Liquid-holding recovery in buffer after X-ray exposure (which does not occur in haploids) is believed to be controlled in part by a MAT-dependent process (Hunnable and Cox, cited in ref. [23]). Moreover, Game and Mortimer [43] have found that some mutants (rad50, rad57) in the RAD52 pathway for repair of ionizing radiation damage exhibit a MAT effect (a/α more resistant than a/a or α/α), while others (rad52, rad54) do not. Thus, RAD52 and RAD54 may act prior to MAT-dependent repair steps, whereas RAD50 and RAD57 may control subsequent MAT-independent steps [43]. A MAT effect for MMS sensitivity has also been reported [106].

Although UV survival does not show the MAT effect [86], UV-induced mitotic recombination does [36]. Frequencies of induced mitotic gene conversion in a/a or α/α diploids were as much as 100-fold lower than in a/α strains, again suggesting that the a/α regulatory state is required for maximal expression of induced mitotic recombination.

There is evidence that MAT regulation can affect UV mutagenesis. Martin, et al. [111] have found that although mms3 causes UV sensitivity in haploids and diploids, a/α mms3/mms3 diploids exhibit defective UV reversion of arg4-17 or lys2-1, compared with a/α mms3/MMS and a/α MMS/MMS diploids; mms3 haploids have wild-type UV revertibility. In addition, a/a or α/α derivatives of the a/α mms3/mms3 strain were restored to normal UV mutability. This shows that the a/α genetic configuration is responsible for the diploid-specific defective UV mutability.

Finally, α umr7-1 haploids fail to express several α-specific haploid functions (such as mating ability, α-factor production, a-factor response), while at the same time they have apparently turned on some a-specific functions ("shmoo" morphology, α-factor proteolysis [34, 35]); on the other hand, a umr7 haploids express normal a-specific functions [104, 105]. Both types of umr7 strains are enormously flocculent (clumpy) but can be dispersed by distilled-water washing [100]. Although these strains are defective in UV mutagenesis at CAN1 [100], it seems likely that this is due to an aberrant cell envelope which interferes in some way with normal expression of mutant arginine permease. This is supported by the observation that non-clumpy revertant derivatives exhibit wild-type levels of UV mutability at CAN1 [104].

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TABLE 1

YEAST MUTANTS SELECTED FOR HYPERSENSITIVITY TO MUTAGENS

Author, year [ref.]	Enrichment (pre-screen)	Isolation (screen)	Genetic loci[a]	Cumulative total (new loci)
Nakai and Matsumoto, 1967 [122]	UV	—[b]	rad1	1
	UV	UV	rad2,rad51	3
Snow, 1967 [159 and unpublished]	EMS	UV	rad1,...,5,10,14	8
Cox and Parry, 1968 [22]	EMS, UV	UV	rad1,...,17,19,...,22,50	23
Resnick, 1969 [142]	NA	UV,X[c]	rad1,2,18,52,53,xs3	27
Moustacchi, 1969 [120]	UV	UV	rad1	27
Zakharov et al., 1970 [179]	UV	UV	rad2,rad4,uvs2	28
Suslova and Zakharov, 1970 [162]	—	X	rad50,51,54,xrs2,xrs4	31
Averbeck et al., 1970 [2]	MNNG	UV	rad2,rad9,r_1^s	32
Snow [unpublished]; Mortimer [unpublished]; Game and Mortimer, 1974 [42]	—	X	rad5,9,18,50,52,...,57	35
Prakash and Prakash, 1977 [137]	UV	MMS	rad1,4,6,52,55,57, mms1,...,22	57
Ananthaswamy et al., 1978 [1]	MNNG	X	rad5,17,50,53,54, + 15 new isolates	61-72

[a]Standardized rad locus assignments are based upon interlaboratory allelism tests [37, 38 39]; rad50 and higher confer only ionizing radiation sensitivity; rad1,..., rad49 have been reserved for those that confer only UV sensitivity or sensitivity to both UV and ionizing radiation; others have not been tested for complementation or assigned to rad loci; ellipses (...) refer to consecutive locus numbers implied by the series.

[b]Identified in a survey of auxotrophic yeast stocks.

[c]Screened for sensitivity to UV or X-rays or both.

TABLE 2

YEAST MUTANTS SELECTED FOR ALTERED MUTAGENESIS OR MITOTIC RECOMBINATION

Author, year [ref.]	Selection phenotype	Genetic loci
Lemontt, 1971 [93]	Decreased UV reversion of arg4-17 (i.e. UV hypo-mutable)	rev1, rev2, rev3
Lemontt, 1973 [96]; 1977 [100]	Decreased UV forward mutation of CAN1 (UV hypo-mutable)	umr1,...,umr7
Rodarte-Ramon and Mortimer, 1972 [149]	Decreased X-ray-induced gene conversion at arg4 (X-ray hypo-rec)	rec1,...,rec5, 2D11, 2C16
von Borstel et al., 1973[167]; Hastings et al., 1976 [55]	Increased spontaneous reversion of lys1-1 (mutator)	mut1,...,mut5,MUT6, mut9,mut10
Maloney and Fogel, 1976 [108]	Increased spontaneous gene conversion at arg4 (hyper-rec)	—
Golin and Esposito,1977 [46]	Increased spontaneous mutation of CAN1 (mutator)	rem1
Quah et al., 1977 [140]	Decreased spontaneous reversion of lys1-1 (antimutator)	—

TABLE 3

ALLELE-SPECIFIC CONTROL OF UV MUTAGENESIS BY REV GENES[a]

Type	Allele	Codon			UV Revertibility			
		Mutant	Normal	Position	REV	rev1	rev2	rev3
Ochre	cyc1-9	UAA	GAA	2	+	-	-	-
	-2	UAA	CAA	21	+	-	+	
	-72	UAA	GAA	66	+	-	+	
Amber	cyc1-179	UAG	AAG	9	+	-	+	-
	-84	UAG	UGG	64	+	-	+	
	-76	UAG	GAG	71	+	-	+	
Initiation	cyc1-131	GUG	AUG	-1	+	+	+	(-)
	-133	AGG	AUG	-1	+	-	+	-
	-13	AUPy	AUG	-1	+	-		-
	-51	CUG	AUG	-1	+	-		-
Frameshift	cyc1-183	+A	AAA	10	+	+	+	-
	-239	-G	AAG	4	+	+	+	-
	-331	-A	GAA	2	+	+		-
Proline missense	cyc1-115	CCPy	CUPy	14	+	+	+	(-)
	-6	CCU	GCU	12	+	-		-

[a]Data from Lawrence and Christensen [87 — 89, 91].

TABLE 4

PRE-REPLICATIVE REPAIR OF HZ-INDUCED PRE-MUTATIONAL DAMAGE[a]

Post-treatment medium[b] (time at 30°C)	Mutation frequency (can1/10^7 viable cells)	
	Control[c]	HZ[c]
None	13.2	15.2
Y (3 h)	24.0	32.0
YCH (3 h)	9.5	14.5
Y (1 day)	9.6	159
YCH (1 day)	12.5	24.7
Y (3 h) + Y (1 day)	12.1	160
YCH (3 h) + Y (1 day)	15.5	88.0
Y (1 day) + Y (1 day)	12.8	174
YCH (1 day) + Y (1 day)	14.0	22.3

[a]Data from Lemontt [102].

[b]Y (YEPD) or YCH (YEPD + 1 μg/ml cycloheximide).

[c]Control or HZ treatment in neutral buffer; 0.2 M, 1 h.