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Deoxyribonucleic Acid Repair in the Yeast *Saccharomyces cerevisiae*

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INTRODUCTION

The yeast *Saccharomyces cerevisiae* has long been an organism of interest to investigators studying cellular responses to deoxyribonucleic acid (DNA) damage. Nearly 40 years ago, Laterjet and Ephrussi (134) correlated sensitivity to radiation killing with the level in ploidy in yeast cells, thus providing one of the earliest indications that the genome is a radiation-sensitive target. The first radiosensitive yeast mutants were not isolated until the late 1960s, and extensive genetic analysis of DNA repair in yeast cells did not begin until the early 1970s. The demonstration of phenotypic and genotypic transformation of yeast cells by DNA-mediated transfection (90) and the construction of a variety of shuttle vectors for gene cloning (16, 256, 258) established yeasts as a versatile eucaryotic paradigm for molecular studies. The availability of an extensive genetic framework coupled with the power of recombinant DNA technology provided incisive tools for probing the complexities of DNA repair, and the past decade has witnessed impressive progress towards understanding the molecular mechanisms by which *S. cerevisiae* responds to genomic injury. There are also encouraging indications that this organism may be a highly informative model for some aspects of cellular responses to DNA damage in higher eucaryotes.

The story is still far from complete and much of what I euphemistically label as progress carries in its wake hints of interesting and unexpected biochemical complexities that promise to challenge students of DNA repair for years to come. Nonetheless, it is useful (and perhaps even necessary)

to periodically review rapidly emerging fields, if only to place recent discoveries in some sort of ordered perspective and to evaluate possible avenues for further research. With these goals in mind, this review is primarily focused on recent advances in cellular responses to DNA damage in *S. cerevisiae*, since the literature already contains several excellent reviews that cover the literature up to about 1980 (64, 65, 80–82, 135, 137, 144, 175, 206, 269, 270). Since that time a number of yeast genes involved in cellular responses to DNA damage have been isolated by molecular cloning, and I have attempted to direct particular attention to information gleaned from such studies.

As a final caveat, it is to be noted that I have emphasized the process of nucleotide excision repair of DNA. (Nucleotide excision repair of DNA is defined as the process[es] whereby damaged bases are excised from the genome as part of an oligonucleotide structure. This process is distinct from base excision repair, in which damaged bases are excised from the genome as free bases [see reference 55].) Aside from my parochial interest in this topic, it remains one of the better understood areas of DNA repair in *S. cerevisiae*. Other recent reviews on cellular responses to DNA damage in yeasts and in other organisms are cited here for the sake of completeness (36, 55–63, 173, 271, 273, 274, 278). The interested reader is also referred to some of the articles mentioned above for discussions of mutagenesis in yeasts, a topic that is not systematically addressed in this review.

GENETICS OF DNA REPAIR IN *S. CEREVISIAE*

The first formal search for yeast mutants abnormally sensitive to killing by ultraviolet (UV) radiation was reported by Nakai and Matsumoto in 1967 (178), following a survey of auxotrophic strains in the Berkeley yeast culture collection. Subsequently, Snow (254) and Resnick (212) independently isolated further UV-sensitive mutants, and Resnick (212) also isolated a series of X-ray-sensitive mutants, some of which were shown to be allelic with recombination-defective mutants previously characterized by Rodarte et al. (229).

In 1968, in what must now be recognized as a seminal contribution to the genetics of DNA repair in yeasts, Cox and Parry (39) reported attempts to saturate the genes of *S. cerevisiae* required for "dark" repair of UV radiation damage (i.e., repair other than photoreactivation), with mutations. A total of 96 mutants were isolated, 24 of which were segregated following back-crosses to the UV-resistant parental strain. Complementation analysis of these 24 mutants demonstrated 14 distinct complementation groups. Of the remaining 76 mutants originally isolated, 37 fell into the 14 established complementation groups. Analysis of the remainder expanded the number of complementation groups to 22. Mutations from each of these could be segregated in systematic pairwise crosses, indicating that each complementation group reflects an independent genetic locus. Of the 22 genetic loci identified in this study, only 12 were represented by more than a single mutant, leading Cox and Parry (39) to argue on statistical grounds that there could be as many as 20 additional loci affecting UV sensitivity, for which no mutant alleles were found.

The large number of genes identified in this study, together with the complexity of the phenotypes of the corresponding mutants, suggested the presence of multiple pathways for the repair of UV radiation damage to DNA. This suggestion was reinforced by the isolation of further mutants by a number of other investigators (6, 132, 133, 141, 172, 295). A systematic nomenclature for radiation-sensitive mutants was established at the International Conference on Yeast Genetics held in Chalk River, Ontario, in 1970. It was agreed that all mutants abnormally sensitive to killing by radiation should be designated as *rad*, with identifying locus and allele numbers. Locus numbers 1 to 49 refer to genes which primarily affect sensitivity to UV radiation or to both UV and ionizing radiation. Locus numbers >50 designate genes which primarily affect sensitivity to ionizing radiation. Game and Cox (66) carried out a series of allelism tests on UV-sensitive mutants from different laboratories and established the independent loci *RAD1* through *RAD22*. In a separate study of X-ray-sensitive mutants, Game and Mortimer (69) established the loci *RAD50* through *RAD57*.

Since the above-mentioned studies, new radiation-sensitive mutants have been identified (115, 203), as well as mutants selected on the basis of sensitivity to monofunctional alkylating agents such as methyl methanesulfonate (MMS) (*mms* mutants) (205) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (187) and to chemicals which cross-link DNA, such as photoactivated psoralen (*pso* mutants) (84) and nitrogen mustard (234, 235). In addition, mutants have been selected for altered levels of spontaneous and induced (by DNA-damaging agents) mutation (65, 71, 79, 138–143, 209, 272) and for altered levels of spontaneous and induced mitotic recombination (230). These include the *rev* mutants, shown to be defective in UV-induced revertibility of various mutant alleles, and the *umr* (UV mutation-resistant) mutants.

Although many of these mutant strains have been shown to carry mutations at distinct genetic loci, the precise allelic relationships of all of these mutants remain to be established (64, 82). At the present time students of DNA repair in yeasts are faced with a large collection of mutant strains that are abnormally sensitive to killing by exogenous physical and chemical agents, and understandably there have been no recent attempts to further saturate the genome with new mutations. This situation is regrettable, since as indicated above there is good reason to believe that not all genes involved in DNA repair in *S. cerevisiae* have been identified (39) and the current incomplete definition of the genetics of sensitivity to DNA-damaging agents may yet prove to limit our understanding of the molecular biology and biochemistry of DNA repair in yeasts.

In a thoughtful and insightful review written for the proceedings of the 1974 UCLA Conference on Molecular Mechanisms for Repair of DNA, Haynes (80) summarized the state of the art of yeast DNA repair and highlighted a number of important goals for the future, two of which merit special consideration here: (i) understanding the functional relationships between the many *RAD* genes identified by mutational analysis; (ii) unravelling the biochemical pathways in which the products encoded by the multiple *RAD* genes presumably participate.

Fundamental information pertinent to understanding the functional relationships of the *RAD* genes emerged from studies on the relative sensitivity of single and double mutants to killing by DNA-damaging agents (26, 32, 38, 40, 44, 65, 67, 83, 86, 138, 142, 206, 209, 249). These studies facilitated the organization of yeast DNA repair genes into different epistasis groups, which, in general, have served as a useful indicator of probable functional relationships. In cases in which the sensitivity of a double mutant is no greater than that of the more sensitive single mutant, the two genes in question are considered to be epistatic (Fig. 1). On the other hand, genes are placed in different epistasis groups

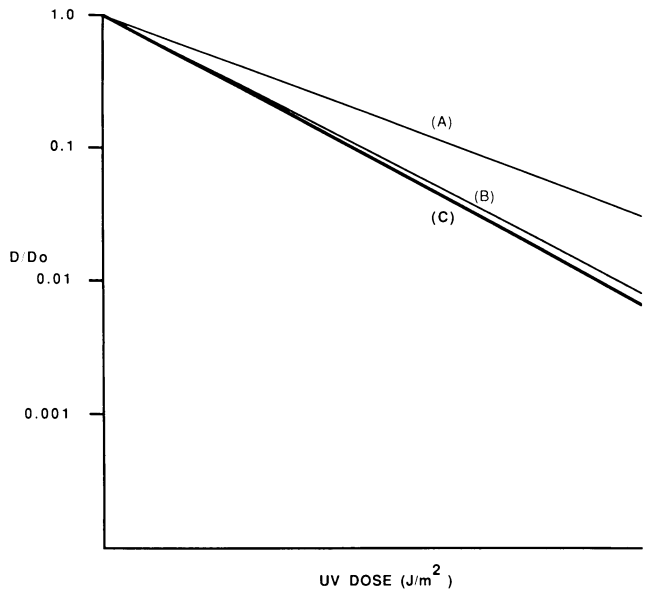

FIG. 1. Diagrammatic representation of an idealized result obtained by analysis of the UV sensitivity of two mutants in the same epistasis group. Lines A and B show the sensitivity of the single mutants *a* and *b*, respectively. Line C shows that the sensitivity of the double mutant (*ab*) is no greater than that of the more sensitive of the two single mutants (*b*). Hence the wild-type genes *A* and *B* are said to be epistatic.

if double mutants show additive or synergistic sensitivity relative to single mutants (Fig. 1). (See Haynes and Kunz [82] for a detailed analysis of additive and synergistic interactions between mutant loci.)

Approximately 30 mutant loci have been so analyzed following exposure of cells to UV or ionizing radiation and have been placed into three epistasis groups referred to as the *RAD3*, *RAD52*, and *RAD6* groups, after prominent mutant loci in each (64, 82) (Table 1). The list of genes in Table 1 has been expanded by phenotypic analysis of some mutants, suggesting that they belong in one of the epistasis groups shown. These three groups of genes are thought to reflect three largely nonoverlapping primary cellular responses to ionizing and UV radiation damage to DNA in the yeast *S. cerevisiae*. Thus, loci in the *RAD3* epistasis group are involved in nucleotide excision repair and those in the *RAD6* epistasis group are required for mutagenesis, while those in the *RAD52* epistasis group are thought to reflect the existence of recombinational responses to DNA damage (see reference 55 for a general review of these DNA repair modes). The simplest interpretation of epistatic interactions between different genes is that they are involved in sequential steps of a multistep biochemical pathway or that they are components of a multimeric or multiprotein complex involved in DNA repair.

As indicated above, this analysis primarily reflects the results of studies with radiation-induced damage to DNA. However, many *rad* mutants are also sensitive to chemicals (82), and a comprehensive determination of epistatic interactions following treatment with such agents has not been achieved. Indeed, studies on the sensitivity of mutants in the *RAD3* epistasis group to simple alkylating agents suggest that the repair of at least some types of monofunctional alkylation damage involves subsets of genes involved in the repair of UV radiation damage (37). Thus, *rad1*, *rad2*, *rad4*, and *rad14* mutants are abnormally sensitive to killing by ethylating agents. However, *rad3*, *rad10*, and *rad16* mutants (which also belong to the *RAD3* epistasis group; Table 1) are not (37).

The situation is further complicated by the observation that different mutants of the *RAD3* epistasis group are sensitive to different ethylating agents. *rad1-1* and *rad2-1* mutants are abnormally sensitive to diethylsulfate, ethylnitrosoguanidine, and ethylnitrosourea, but not to ethyl methanesulfonate. On the other hand, *rad4-4* and *rad14-2* mutants are sensitive to ethylnitrosoguanidine and ethylnitrosourea, but are resistant to diethylsulfate (37). Finally, sensitivity to many of the agents tested is not confined to *RAD3* epistasis group mutants (37). Hence, if the epistatic interactions of the mutant loci listed in Table 1 were reexamined with respect to monofunctional alkylation damage, a different classification of these genes might be expected. Indeed, von Borstel and Hastings (270) have appropriately suggested that the various epistasis groups should not be viewed as repair pathways, but rather as repair systems which can be reassorted for different purposes.

In summary, the *RAD* epistasis groups represent a convenient way of establishing the genetic complexity of cellular responses specifically to UV and to ionizing radiation damage in *S. cerevisiae*. In addition, they are useful for defining probable functional relationships between genes and in at least one case for defining a specific repair pathway; i.e., the *RAD3* epistasis group appears to genetically define the nucleotide excision repair pathway for repair of UV radiation damage (see later).

TABLE 1. Epistasis groups for yeast genes involved in cellular responses to DNA damage[a]

RAD3	*RAD52*	*RAD6*
CDC8	*CDC9*	**CDC7*
CDC9	*RAD24*	*CDC8*
MMS19	*RAD50*	*CDC40*
PSO2 (SNM1)	*RAD51*	*MMS3*
RAD1	*RAD52*	**NGM2*
RAD2	*RAD53*	*PSO1*
RAD3	*RAD54*	**RAD5 (REV2) (SNM2)*
RAD4	*RAD55*	*RAD6*
RAD7	*RAD56*	**RAD8*
RAD10	*RAD57*	*RAD9*
RAD14	*PSO1*	**RAD15*
RAD16		*RAD18*
RAD23		*REV1*
RAD24		*REV3*
UVS12		**REV5*
		**REV6*
		REV7
		**UMR1*
		**UMR2*
		**UMR3*

[a] The genes are shown as wild-type alleles for simplicity. However, all analyses bearing on epistatic assignments were of course carried out with mutant strains. The assignment of genes to a particular epistasis group is not based on a comprehensive analysis of UV or ionizing radiation sensitivity in all cases. In fact, in some cases (*) genes have been assigned strictly on the basis of phenotypic characterization. Analysis of mutant derivatives of the genes shown in parentheses indicates that they are allelic to those primarily listed. The table is based primarily on that of Haynes and Kunz (82) and has been updated with information supplied by Bernard Kunz, Ethel Moustacchi, and Wolfram Siede.

RAD3 EPISTASIS GROUP

Radiation Biology

Mutations at all loci in the *RAD3* epistasis group (Table 1) confer varying levels of sensitivity to UV radiation (see reference 82). As just indicated, sensitivity to chemical agents which damage DNA, particularly those generally considered to be UV mimetic, has not been systematically explored. However, some mutants in this epistasis group have been reported to show moderate sensitivity to ionizing radiation or monofunctional alkylating agents (see above) and marked sensitivity to nitrogen mustard or photoactivated psoralens (see reference 82). In fact, recent studies have provided evidence for a role of the *RAD3* group of genes in the repair of DNA damage produced by X rays. Specifically, it has been shown that double mutants defective in genes in the *RAD6* and *RAD52* epistasis groups are less sensitive to killing by X rays than triple mutants which also contain a mutation in one of the *RAD3* group genes (J. C. Game, D. Schild, and R. K. Mortimer, personal communication). This suggests the existence of a form of X-ray damage to DNA that can be repaired by pathways represented by all three epistasis groups and hence does not contribute to lethality unless genes in all three groups are mutated.

In view of the rather limited information on repair of chemical damage to DNA in yeast, this discussion of the *RAD3* epistasis group focuses primarily on UV radiation damage as a model for phenotypic characterization of the *RAD3* group mutants. Mutations in the *RAD1*, *RAD2*, *RAD3*, *RAD4*, and *RAD10* genes confer marked sensitivity to killing by UV radiation (39), while *rad7*, *rad14*, *rad16* (39), *rad23* (cited in reference 82), *rad24* (6, 44), *cdc8* (203), and *mms19* (207) mutants are considerably less UV sensitive. Among the group of highly sensitive mutants (referred to henceforth as the *rad1*, etc., group), all three *rad4* mutants carrying point mutations (*rad4-2*, *rad4-3*, and *rad4-4*) as well as the two *rad10* mutants carrying point mutations (*rad10-1* and *rad10-2*) are less UV sensitive than the *rad1*, *rad2*, or *rad3* strains with similar mutations, though considerably more sensitive than the *rad7*, etc., group. The recent cloning of the *RAD4* and *RAD10* genes (see later) facilitated the construction of disruption and deletion mutants and led to the demonstration that the *rad4* and *rad10* mutations are in fact leaky (53, 279). In some alleles this leakiness is apparently due to partial suppression of ochre (*rad4-3*) and amber (*rad10-1*) mutations by the normal chromosomal transfer ribonucleic acid (tRNA) genes $tRNA^{Gln}_{CAA}$ and $tRNA^{Gln}_{CAG}$, respectively (208, 279, 281).

The demonstration that carefully selected strains with mutations in these five *RAD* genes are approximately equally (and highly) sensitive to killing by UV radiation is consistent with their apparent absolute requirement for the excision of pyrimidine dimers (see later). Whenever possible, therefore, the phenotypic characterization of radiation-sensitive yeast strains should be carried out with deletion or disruption mutants rather than with point mutants. In cases in which the gene in question has been cloned, such nonleaky mutants can be readily constructed by reverse genetics.

The observation that some *rad* alleles are leaky raises obvious questions concerning the interpretation of the phenotype of the *rad7*, etc., group of mutants. In the case of the *rad7* and *rad23* mutants, it has been established that leakiness is not an explanation for their limited UV sensitivity, since deletion mutants have the same phenotype as point mutants (167, 196). While comparable evidence for the *rad14*, *rad16*, and *mms19* mutants must await the isolation of alleles with disruptions or deletions (the *RAD24* and *CDC8* genes are essential and hence would be inviable if disrupted), it is clear that some *RAD3* epistasis group mutants have a distinct phenotype of moderate sensitivity to UV radiation, and this must ultimately be reconciled with their role in the repair of UV radiation damage to DNA.

Characterization of Defective DNA Repair

The phenomenon of liquid holding recovery in yeast cells was established by studies in the early 1960s. Several investigators demonstrated that cultures of UV-irradiated cells held in non-nutritive suspension prior to plating showed enhanced recovery relative to cells not held under such conditions (120, 193–195). Similar results were obtained earlier with bacteria (75, 227), and the observation that liquid holding recovery was not detected in excision-defective bacterial mutants suggested that the phenomenon in yeasts also reflects (at least in part) the operation of dark repair mechanisms. Soon after the isolation and preliminary genetic characterization of UV-sensitive mutants by Cox and Parry (39), it was demonstrated (191) that some *RAD3* epistasis group mutants were defective in liquid holding recovery. The additional observation that survival of these mutants could be increased by exposure of UV-irradiated cells to photoreactivating conditions led to the suggestion that these mutants were defective in pyrimidine dimer excision.

A role for genes in the *RAD3* epistasis group in nucleotide excision repair of DNA is consistent with studies on UV-induced mutagenesis. A number of investigators have shown that mutants carrying this group of genes consistently show higher levels of UV radiation-induced mutations than do wild-type cells (43, 98, 121). This is precisely what one would expect from mutation inactivation of a largely error-free cellular response to DNA damage.

Direct studies on excision repair of pyrimidine dimers were hampered initially by the lack of strains in which DNA could be specifically labeled in thymine, thus facilitating the quantitation of thymine-containing pyrimidine dimers. *S. cerevisiae* does not possess thymidine kinase activity (72); hence, radiolabeled thymine or thymidine is not metabolized to deoxythymidine 5′-triphosphate. Unrau and his colleagues (265) labeled UV-irradiated cells with radioactive uracil, and following the purification of high-molecular-weight DNA, they chromatographically resolved radiolabeled uracil-uracil, uracil-thymine, and thymine-thymine dimers. Direct quantitation of these photoproducts in Rad^+ and *rad1-1* cells showed that the latter (*RAD3* epistasis group mutants; Table 1) are defective in the excision of pyrimidine dimers (265). This result was confirmed by others (276) in both exponential and stationary cultures. These were the first direct biochemical demonstrations of defective nucleotide excision repair of DNA in *S. cerevisiae*, and by inference it was assumed that other members of the *RAD3* epistasis group were also defective in this process.

Incidentally, it should be mentioned that the ability to quantitate pyrimidine dimers in DNA led to the calculation that the dose of UV radiation required to reduce the survival of Rad^+ cells to 37% (D_{37}) results in ~27,000 pyrimidine dimers per haploid genome (38). All three epistasis groups of genes apparently participate in cellular responses to the presence of pyrimidine dimers in DNA, since mutant strains with blocks in all three groups manifest exponential UV survival curves from which it can be calculated that, on the

average, one to two pyrimidine dimers per haploid genome are lethal (38).

Further evidence in support of a defect in the excision repair of pyrimidine dimers in mutants defective in the *RAD3* group of genes came from an elegant exploitation of the specificity of photoreactivating enzyme for pyrimidine dimers in DNA. It was shown that, when UV-irradiated Rad$^+$ cells were incubated in the dark, sites reactivatable with photoreactivating enzyme during subsequent incubation of their DNA (i.e., pyrimidine dimers) were progressively lost. This dark-dependent loss of photoreactivatable sites was not observed with UV-irradiated *rad2* cells, however (217).

At about this time the problem of specific radiolabeling of DNA in *S. cerevisiae* was solved by the isolation of strains that can take up thymidine monophosphate and incorporate it into their genome (25, 48, 105, 287) and later by the isolation of authentic thymidine monophosphate auxotrophs (27, 48, 145). However, the use of these mutants for measuring excision of pyrimidine dimers would require that the thymidine monophosphate-defective genotype be systematically introduced into all *RAD3* epistasis group mutants, a labor-intensive task that may not be compatible with viability in all cases (for example, there are indications that a *rad6* strain that can take up deoxythymidine monophosphate is inviable [M. Brendel, unpublished observations]).

By the mid-1970s, more sensitive techniques for measuring nucleotide excision repair were in vogue. These utilized the direct measurement of strand breaks in the nuclear DNA of UV-irradiated cells as a function of postirradiation incubation time or incubation of DNA from UV-irradiated and incubated cells with pyrimidine dimer-specific enzyme probes isolated from *Micrococcus luteus* or *Escherichia coli* cells infected with bacteriophage T4 (198, 223). Both of these techniques involve sedimentation of DNA in alkaline sucrose gradients; thus, RNA is degraded and the use of a general metabolic label such as [^{3}H]uracil is quite satisfactory.

Analysis of DNA strand breaks introduced directly by incision of DNA during nucleotide excision repair has limited sensitivity because at any given moment the number of breaks that can be detected by sedimentation of DNA is very small. This presumably reflects the operation of a processive mode of excision repair in yeast cells. Processive excision repair of DNA has been demonstrated in vitro with the phage T4 pyrimidine dimer-DNA glycosylase/AP-endonuclease (70, 73, 147) and has been suggested for the *E. coli* UvrABC enzyme (see reference 58). Such processivity has not been directly demonstrated in yeast cells, however. Despite the limited sensitivity of this technique, Reynolds and Friedberg (224, 225) were able to show that a number of *rad1*, *rad2*, *rad3*, and *rad4* mutants were defective in the incision of UV-irradiated DNA in vivo.

The introduction of strand breaks with dimer-specific endonuclease probes into DNA isolated and purified following the irradiation and incubation of cells is more sensitive for evaluating defective excision repair than the direct measurement of DNA strand breaks (198). In essence, this technique relies on the fact that, if pyrimidine dimers were removed from the genome by excision repair in vivo, sites sensitive to dimer-specific probes are lost and hence are not converted to strand breaks detected by sedimentation velocity. However, the sensitivity of this technique is severely compromised if a large number of dimers are introduced into the genome. When many dimers are converted to DNA strand breaks, the weight-average molecular weight of the DNA is too small to resolve minor differences in dimer content (i.e., the loss of enzyme-sensitive sites) by sedimentation velocity.

In an attempt to overcome this limitation, Wilcox and Prakash (288) modified the direct measurement of strand breaks generated de novo during postirradiation incubation of cells. By introducing the *cdc9* mutation (see later) into yeast strains of interest, they established a mechanism for inhibiting DNA ligase activity. Hence, a larger fraction of the incisions generated during nucleotide excision repair in vivo could be detected by sedimentation velocity because the cells could not complete the ligation step.

With the use of the various experimental strategies discussed above, it has been shown that cells carrying mutations in the *RAD1*, *RAD2*, *RAD3*, *RAD4*, and *RAD10* genes do not carry out detectable incision of their DNA during postirradiation incubation, thereas those carrying mutations in the *RAD7*, *RAD14*, *RAD16*, *RAD23*, and *MMS19* genes have a significant residual capacity for incising DNA at sites of pyrimidine dimers (159, 200, 201, 207, 224, 225, 288). As indicated earlier, mutants that include deletions of the entire *rad7* and *rad23* genes are only partially defective in excision repair of pyrimidine dimers (196). Hence, it seems reasonable to conclude that the partial defects exhibited by the *rad14*, *rad16*, and *mms19* mutants are not fortuitous and leads me to the viewpoint that these members of the *RAD3* epistasis group may not be primarily involved in the incision of DNA at sites of pyrimidine dimers (and by inference at sites of other forms of bulky base damage), but may have a secondary role in this process.

At present, one can only speculate on the nature of such a secondary role. Recent information on the molecular mechanism of excision repair in *E. coli* suggests possible clues. *uvrA*, *uvrB*, or *uvrC* mutants are severely defective in nucleotide excision repair and, like the *rad1*, *rad2*, *rad3*, *rad4*, and *rad10* yeast mutants, are highly sensitive to killing by UV radiation (see references 55 and 56). However, *uvrD* and *polA* mutants are less sensitive to killing by UV radiation and show a residual capacity for dimer excision in vivo (see references 55 and 56). Studies with purified Uvr proteins indicate that, qualitatively, incision of UV-irradiated DNA requires only the UvrA, UvrB, and UvrC proteins (see references 55 and 56). However, in the absence of other proteins, the extent of DNA incision and the excision of oligonucleotides containing pyrimidine dimers is limited (31, 99, 123). Addition of UvrD and PolI proteins increases turnover of the UvrABC complex, thereby facilitating further incision and excision events (31, 99, 123). Hence, the overall kinetics of excision repair in vitro is improved in the presence of these two proteins, and this suggests that the role of the UvrD and PolI proteins in nucleotide excision repair is quantitative rather than qualitative (56). Extrapolating to yeasts, one might argue that the *RAD1*, *RAD2*, *RAD3*, *RAD4*, and *RAD10* genes are essential for qualitative events during excision repair of bulky base damage and that the *RAD7*, etc., genes play a kinetic (i.e., quantitative) role in this process (56).

The distinction between genes with primary and secondary roles in nucleotide excision repair provides a reasonable explanation for the genetic complexity of this process in yeasts. However, other (not necessarily exclusive) explanations for this complexity merit consideration. It has been suggested that in eucaryotes the association of DNA with histones and with nonhistone chromosomal proteins, together with the various levels of coiling of the genome inherent in the structural organization of chromosomes, may present special problems for the access of catalytically

active repair enzymes to sites of base damage (see reference 55). This problem may in part be accommodated by the general accessibility provided during transcription of the genome. Indeed, there is recent compelling evidence that in mammalian cells excision repair may occur preferentially in actively transcribing genes (22, 23, 149). Also, or alternatively, cells may have the specific capacity for "opening up" the genome during DNA repair, an attractive role for some of the many proteins apparently involved in nucleotide excision repair in yeasts.

A final thought concerning the involvement of multiple proteins in excision repair stems from recent speculations by Echols (42). This author has pointed out that many DNA transactions require a high degree of specificity for DNA-protein interactions. One mechanism for achieving this is to utilize specialized multiprotein-DNA complexes, the three-dimensional structure of which provides the biochemical basis for the specificity of the required interactions.

Plasmids To Monitor Nucleotide Excision Repair

In addition to the direct study of nuclear DNA (there is no evidence for excision repair of damage from mitrochondrial DNA in *S. cerevisiae* [175, 198, 277]), a number of laboratories have investigated the use of plasmids for evaluating excision repair in yeast cells. Most *S. cerevisiae* strains normally contain about 50 to 100 copies of a double-stranded DNA plasmid. The contour length of this plasmid is ~2 μm, and hence it is frequently referred to as the yeast 2μm plasmid (253). The plasmid is in many ways a model mini-chromosome. It is replicated at a specific time in the cell cycle (296) and is physically associated with histones (146).

McCready and Cox (160) isolated 2μm plasmid DNA from unirradiated and UV-irradiated cells incubated for varying periods of time to allow excision repair. After a dose of 20 J/m^2, ~86% of the plasmid molecules acquired sites sensitive to nicking by the *M. luteus* dimer-specific endonuclease, and following a 3-h incubation essentially all enzyme-sensitive sites were removed. Consistent with excision repair in nuclear DNA, plasmids isolated from a *rad1* mutant showed no significant loss of dimers, even though plasmid-borne dimers in this strain were repairable by photoreactivation. More recently, McCready et al. (159) extended these studies to include other mutants in the *RAD3* epistasis group. As expected from studies on repair in nuclear DNA, *rad2*, *rad3*, and *rad4* mutants showed no detectable repair of plasmid DNA, whereas *rad7* and *rad14* mutants showed residual repair. Surprisingly, McCready et al. (159) reported no detectable repair of plasmid DNA in a *rad16* mutant, despite the fact that in all reported studies (including their own) this strain repaired nuclear DNA to a significant extent.

A related study was carried out by Dominski and Jachymczyk (41), who observed normal levels of repair of plasmid DNA in a mutant strain carrying the *rad3-2* allele, an unexpected result which is contrary to that observed in the nuclear DNA of this strain. In this study, the 2μm-based plasmid also contained an origin of replication for propagation in *E. coli*, and when tested in an *E. coli uvrA* mutant no repair of plasmid DNA was detected. Hence, there was no indication that lesions in this plasmid were generally refractory to excision repair. Unfortunately, other *rad3* mutant alleles were not examined by these authors. Similarly surprising results were reported by Ikai et al. (101), who measured transformation frequencies for various *rad* mutants transfected with a UV-irradiated multicopy plasmid. They noted that, relative to a wild-type strain, transformation was unaffected in the *rad3-2* strain and also in *rad1-2*, *rad2-6*, and *rad4-4* strains. However, as in the study by Dominski and Jachymczyk (41), in *E. coli uvrA* the transformation frequency was dramatically reduced relative to that of a *uvrA*$^+$ strain.

White and Sedgwick (282) monitored the survival of UV-irradiated multicopy plasmids after transformation of wild-type and mutant yeast strains. Plasmid DNA showed decreased levels of transformation (i.e., increased sensitivity to UV radiation) in all mutants tested from the *RAD3* epistasis group, including the *rad3-2* strain. Hence, in this study, the survival of irradiated plasmid DNA qualitatively reflected the cellular resistance to UV radiation. White and Sedgwick (282) noted that the UV sensitivity of the *rad3-2* strain reported by Dominski and Jachymczyk (41) was significantly less than that expected and suggested that the *rad3-2* strain used by the latter authors may have accumulated suppressors. However, there is no obvious explanation for the results of Ikai et al. (101), and the use of plasmids for probing the molecular biology of excision repair in *S. cerevisiae* may involve experimental artifacts that remain to be identified.

Further "problems" associated with the use of plasmids surfaced from more recent studies by White and Sedgwick (283). These investigators incubated plasmid DNA with purified UV endonuclease of *M. luteus*, prior to transformation. Treatment of UV-irradiated DNA with this enzyme had little effect on transformation of wild-type strains. In contrast, little or no rescue of transforming efficiency was observed in *rad1-1*, *rad4-4*, *rad7-1*, or *rad14* strains, suggesting that these strains were unable to process incisions generated by the *M. luteus* enzyme. This result is difficult to explain. The mechanism of incision of UV-irradiated DNA by the pyrimidine dimer-specific *M. luteus* (and phage T4) enzymes is distinct from that of the more general *E. coli* UvrABC endonuclease (see reference 55) and, by inference, from that of the putative general yeast nucleotide excision repair enzyme. Thus, if wild-type yeast cells are able to excise dimers from DNA in which incisions were initiated by an exogenous DNA-glycosylase/AP endonuclease, there is no a priori reason to expect that *rad* mutants should be defective in this process. In fact, in experiments with yeast cell extracts, no defect in excision of thymine dimers from preincised (with *M. luteus* enzyme) UV-irradiated DNA was detected in any *rad* mutants examined (226). Furthermore, when various incision-defective *rad* mutants were transformed with a plasmid containing the cloned phage T4 *denV* gene (which encodes the T4 dimer-specific DNA glycosylase/AP endonuclease), partial complementation of the UV sensitivity of these mutants was readily observed, suggesting that the defect in incision of UV-irradiated DNA in vivo can be complemented by this enzyme (33).

Nucleotide Excision Repair in Cell-Free Systems

Contrary to the situation in *E. coli*, studies on nucleotide excision repair of UV-irradiated DNA that use cell-free yeast systems have been limited and unpromising to date. Bryant and Haynes (29) reported the partial purification and preliminary characterization of an endonuclease designated endonuclease α, which preferentially (but not exclusively) nicked UV-irradiated linear T7 DNA. The level of this endonuclease in partially purified extracts of *rad1-1* cells was approximately half that observed in extracts of wild-type cells. However, *rad3-2* cells had normal levels of the enzyme.

Bekker and his colleagues (13) assayed extracts of wild-type and *rad* mutant cells for nicking of unirradiated and UV-irradiated form I plasmid DNA and reported that extracts of all mutants examined showed preferential nicking of the UV-irradiated substrate. Similar results were obtained with DNA irradiated at 340 nm in the presence of the photosensitizer acetophenone, conditions which result in the production of thymine dimers as the major, if not the exclusive, photoproduct (see reference 55). The authors concluded that the *rad1*, *rad2*, *rad3*, *rad4*, *rad10*, and *rad16* mutants were not defective in the incision of UV-irradiated DNA, but suggested that the defect in these mutants might be at the level of postincisional excision events. Consistent with this interpretation, they subsequently reported that extracts of both wild-type and *rad* mutant cells catalyzed the complete excision of pyrimidine dimers from UV-irradiated DNA in vitro (14). Remarkably, in both studies DNA was incubated with cell extracts in the presence of ethylenediaminetetraacetate, suggesting no requirement for divalent cation in the incision or excision reaction(s).

In vitro attempts to reproduce these results have not been successful. Incubation of UV-irradiated DNA or chromatin with extracts of repair-proficient yeast cells did not result in the preferential loss of thymine-containing dimers under a variety of experimental conditions tested in my laboratory (224; J. D. Love and E. C. Friedberg, unpublished observations).

The failure to date to establish a cell-free system in which crude extracts of yeast cells reproducibly catalyze selective incision of damaged DNA or excision of bulky adducts such as pyrimidine dimers, or both, places considerable restraints on future biochemical studies. Regardless of the methods ultimately used to purify the products encoded by genes in the *RAD3* epistasis group, evaluation of the functional integrity of these proteins will require the availability of a cell-free assay. Ideally, such an assay should reflect the ability of selected Rad proteins to complement appropriate mutant extracts for biochemical activities consistent with nucleotide excision repair of DNA and the inability of mutant proteins to do so.

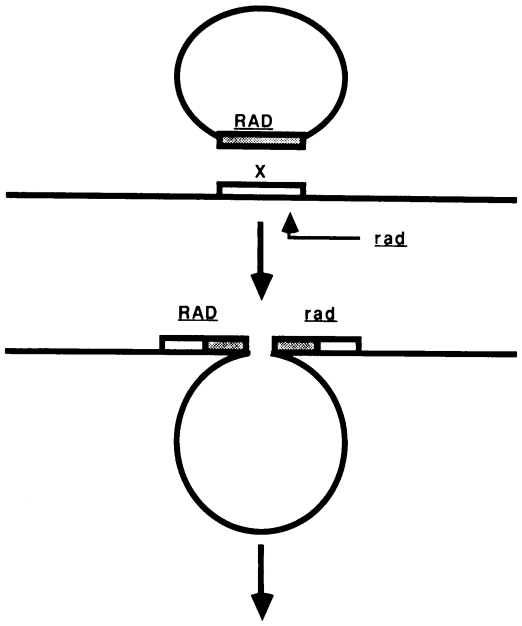

FIG. 2. Diagrammatic representation of a strategy to show that a cloned yeast gene is a particular *RAD* gene of interest. An integrating plasmid carrying a DNA insert that includes the cloned *RAD* gene is transfected into a mutant *rad* strain defective in the gene of interest. Integrants are selected following the stable integration of a marker gene (e.g., *URA3*) carried on the plasmid. These integrants are analyzed for UV resistance. If the plasmid contains the correct *RAD* gene, homologous recombination will result in tight genetic linkage of the *RAD* and *rad* alleles. Thus, when these phenotypically Rad^+ strains are mated to a different Rad^+ strain, the probability of segregating Rad⁻ haploids by meiotic recombination is extremely low. If the cloned gene is not homologous to the *rad* mutant allele, integration will occur at some other site in the genome and the results of the genetic crosses will obviously be quite different.

Molecular Cloning of Genes in the *RAD3* Epistasis Group

The advent of recombinant DNA technology has resulted in considerable progress toward the goal of unravelling the molecular biology and biochemistry of nucleotide excision repair in yeasts. The marked UV sensitivity in the *rad1*, *rad2*, *rad3*, *rad4*, and *rad10* mutants provides an obvious phenotype for the selection of transformants with enhanced UV resistance, and screening of yeast genomic libraries has resulted in the isolation of recombinant plasmids which specifically complement a number of selected *rad* mutant cells.

In all cases refined genetic analysis of the cloned inserts has confirmed that they contain the specific *RAD* genes of interest rather than sequences which nonspecifically complement the phenotype of UV sensitivity. Such genetic analyses are particularly facile in *S. cerevisiae* because transformation of yeast cells with plasmids containing cloned genomic sequences results in integration of the cloned sequences almost exclusively at sites of homology (see reference 61). Hence, it is possible to integrate a cloned wild-type gene in very close physical and genetic proximity to a chromosomal mutant allele and establish the identity of the cloned sequence by phenotypic analysis of the integrant derivative (Fig. 2). These and other strategies for the genetic analysis of cloned yeast genes are discussed in greater detail elsewhere (61). Suffice it to state here that analysis of plasmids isolated by phenotypic complementation of selected *rad* mutants has resulted in the unequivocal identification of the *RAD1* (89, 292, 293), *RAD2* (87, 182), *RAD3* (88, 180), and *RAD10* (202, 280) genes.

Comprehensive screening of several yeast genomic libraries failed to yield recombinant plasmids containing the *RAD4* gene (208). Recently, the *RAD4* gene was isolated by an alternative cloning strategy. The gene is known to be genetically tightly linked to the *SPT2* locus on the right arm of chromosome V (290). The *SPT2* gene has been cloned (231), and an integrating plasmid containing the *spt2-1* mutant allele was shown to complement the UV sensitivity of several *rad4* mutant strains (52). Physical mapping of the *RAD4* gene demonstrated that the entire gene is present in the integrating plasmid containing the *spt2-1* allele (52). However, this as well as all other *RAD4*-containing plasmids propagated in *E. coli* contain inactive alleles (52, 53). These alleles arise by selection of mutationally inactivated forms of the gene because expression of the *RAD4* gene is toxic to *E. coli*.

Screening *rad4* mutants with plasmid libraries constructed from yeast DNA not propagated in *E. coli* yields plasmids with a functional *RAD4* gene (251). Similarly, repair of

TABLE 2. Cloned DNA repair genes from *S. cerevisiae* and the proteins they express[a]

Gene	Size of coding region		Codon bias index	Calculated size of polypeptide (kDa)	Observed M_r of protein by SDS-PAGE ($\times 10^3$)	Calculated net charge of protein at pH 7.0
	bp	Codons				
RAD1	3,300	1,100	0.39	126.2	~150	−36
RAD2	3,093	1,031	0.40	117.7	~150	−35
RAD3	2,334	778	0.42	89.7	~90	−19
RAD4	2,262	754	0.41	87.1		+4
RAD10	630	210	0.40	24.3	~24	+3
RAD7	1,695	565	0.41	63.7		−13
CDC9	2,265	755	0.46	84.8	~88	+1
CDC8	648	216	0.43	24.6	~25	−4
PHR1	1,695	565	0.46	57.2–66.2	~53	+18
RAD50	3,936	1,312		157		
RAD52	1,512	504	0.42	56.1		+3
RAD6	516	172	0.42	19.7	~20	−23

[a] The codon bias indices were calculated as described by Bennetzen and Hall (17). The calculated sizes and net charge of the polypeptides from the sequenced genes shown were derived by computer analysis, using the DNA Inspector II$^+$ program of Textco. SDS-PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

mutant *rad4* alleles by transfer of wild-type genomic information to gapped plasmids (double-strand gap repair of DNA [189, 190]) restores a wild-type *RAD4* gene (52). However, in both situations propagation of the wild-type allele in *E. coli* results in a markedly reduced transformation frequency and all plasmids recovered are extensively deleted or rearranged, or both, or contain point mutations in the *RAD4* locus (52, 53).

Inactivation by insertion of a random DNA fragment into a convenient unique restriction site in the cloned gene facilitates the propagation of plasmid DNA in *E. coli* without further inactivating events (53). Following the removal of the inserted fragment, a functional gene is restored. The reason for the apparent toxicity of Rad4 protein to *E. coli* is unknown. This phenotype presumably reflects one or more biochemical attributes that are apparently unique to this protein among the many Rad proteins now expressed in *E. coli* from cloned genes.

***RAD1* gene.** The *RAD1* gene of *S. cerevisiae* has been cloned in a number of laboratories (89, 292, 293). The sequence of the *RAD1* coding region reveals an open reading frame (ORF) of 3,300 nucleotides (221), the largest of the genes thus far cloned from the *RAD3* epistasis group (Table 2). The gene is expected to encode a protein of calculated M_r 126,200 (Table 2). As is typical of most yeast genes transcribed by RNA polymerase II, there are no intervening sequences in this or any of the other cloned excision repair genes. The translated amino acid sequence does not show significant similarities to other protein sequences in the data banks surveyed (including *E. coli*, human, and other yeast excision repair genes). Hence, the sequence of the *RAD1* gene has not provided obvious clues concerning the nature or possible catalytic function of Rad1 protein.

A *RAD1-lacZ* fusion gene expressed very low levels of β-galactosidase activity relative to that expressed from a *HIS4-lacZ* fusion (56). Since *HIS4* is a strongly expressed yeast gene (252), these data suggest that *RAD1* is normally weakly expressed. This conclusion is supported by direct measurement of the steady-state levels of *RAD1* messenger RNA (mRNA) in yeast cells. Relative to the yeast *URA3* gene (~five copies per cell), it has been estimated that there are only 0.05 to 0.1 copy of *RAD1* mRNA per cell (E. Yang, Ph.D. thesis, Stanford University, Stanford, Calif., 1987). Indeed, it is extremely difficult to detect the 3.8-kilobase (kb) *RAD1* transcript by conventional Northern (RNA) blot analysis. The major transcriptional start site of the *RAD1* gene has been mapped to a position ~110 nucleotides upstream of the first ATG codon in the ORF. Two minor transcripts have been located at positions −50 and +5 (Yang, Ph.D thesis).

In cells transformed with multicopy plasmids containing the cloned *RAD1* gene, ~10 times as much mRNA can be detected relative to untransformed cells. The *RAD1* transcript in these cells is larger (5.7 kb) than that detected in untransformed cells (3.8 kb) and is apparently transcribed from different start sites (at nucleotide positions −155, −315, and −355) (Yang, Ph.D thesis). Unlike the case in bacteria and higher eucaryotes, transcriptional regulatory sequences are difficult to identify unambiguously by visual examination of the nucleotide sequence. The consensus yeast regulatory sequence is TATAAT (24). However, since many yeast genes are very AT rich, particularly in 5′ noncoding regions, similarities to such a consensus sequence can be found quite readily, and their significance must be evaluated with caution.

The observation that *RAD1* is a weakly expressed gene is consistent with the codon composition of the cloned gene. It has been noted that many strongly expressed *E. coli* and yeast genes have a bias for codons homologous to the anticodons of the major isoacceptor tRNA species and are biased against codons for rare tRNAs (17, 92, 247). Correspondingly, weakly expressed genes show no particular bias against codons for rare tRNAs (17, 92, 247). Thus, calculations of the fraction of total codons corresponding to the 22 most frequently used codons in highly expressed yeast genes yield values of 0.9 to 1.0, whereas the same calculations for weakly expressed genes yield values significantly lower. All of the genes analyzed in this review have calculated codon bias indices (as defined above) of ~0.4 (Table 2).

An explanation offered for this correlation is that the requirement for large amounts of particular cellular proteins is met by rapid translation, using abundant tRNAs (17). Rapid translation would presumably be favored by the use of codons for abundant tRNAs, since the concentration of charged cognate tRNAs is rate limiting for the addition of each amino acid to the growing polypeptide chain. Based on this logic, it has been suggested that in genes which encode large amounts of proteins, i.e., those which are highly expressed, there has been selection for codons that utilize abundant tRNAs (17). The sequence of the cloned *RAD1* gene shows no bias for codons corresponding to the anticodons of abundant tRNAs, in support of the contention that this gene is weakly expressed.

TABLE 3. Sequence context in mRNAs of yeast DNA repair genes near the translational start site[a]

Consensus	−7	−6	−5	−4	−3	−2	−1	+1	+2	+3	+4	+5	+6	
			A	A/C	A	A/C	A	**A**	**U**	**G**	U			(Ser)
RAD1	U	U	U	C	C	A	G	**A**	**U**	**G**	U	C	U	(Ser)
RAD2	U	U	C	A	G	A	C	**A**	**U**	**G**	G	G	U	(Gly)
RAD3	U	G	G	A	A	A	C	**A**	**U**	**G**	A	A	G	(Lys)
RAD4	C	G	C	U	A	A	A	**A**	**U**	**G**	A	A	U	(Asn)
RAD10	C	U	A	G	A	A	G	**A**	**U**	**G**	A	A	C	(Asn)
RAD7	G	G	A	A	G	A	A	**A**	**U**	**G**	U	A	U	(Tyr)
CDC8	A	U	U	U	U	G	A	**A**	**U**	**G**	A	U	G	(Met)
CDC9	C	A	A	U	U	A	C	**A**	**U**	**G**	C	G	C	(Arg)
PHR1	A	C	U	G	A	U	A	**A**	**U**	**G**	A	A	A	(Lys)
RAD52	G	U	A	U	C	G	A	**A**	**U**	**G**	G	C	G	(Ala)
RAD6	A	A	G	C	G	U	C	**A**	**U**	**G**	U	C	C	(Ser)

[a] The putative mRNA sequences are derived from the sequenced cloned genes shown. The sequence context analysis is based on that described by Hamilton et al. (74) and includes the consensus sequence derived by them.

Hamilton et al. (74) recently reported a correlation between the sequence context surrounding the AUG start codon in mRNAs and the level of expression of many yeast genes. The AUG start codon context in yeast mRNAs is quite different from that of mammalian genes. Whereas the latter have the consensus sequence $CC^{A}_{G}CCAUGG$, an analysis of 96 yeast sequences revealed the consensus A^{A}_{C} $A^{A}_{C}AAUGU$ (Table 3). Thus, in yeasts the region immediately preceding the translational start codon is very rich in A relative to that in mammalian cells. Nonetheless, in both yeasts and mammals A is the most frequent base at position −3. Hamilton et al. (74) observed that, in ~50% of highly expressed yeast genes examined, G is absent in the seven nucleotides immediately preceding the AUG start codon. Also, A is present at positions −3 and −1 at frequencies of 100 and 89%, respectively, and the codon immediately succeeding the methionine start is the serine codon UCU (Table 3). Table 3 also shows an analysis of the AUG context of the cloned yeast DNA repair genes discussed in this review. It should be noted that, with the single exception of *CDC9*, G is represented in the seven bases preceding the AUG start. Of the 11 sequences shown, A is present at position −3 in *RAD3*, *RAD4*, *RAD10*, and *PHR1*. However, only *PHR1* and *RAD4* also have A at position −1. Finally, with the single exception of *RAD1*, none of these genes use the serine codon UCU after the methionine start (Table 3). Thus, based on the criteria established by Hamilton et al. (74), none of these genes can be considered to be strongly expressed.

The level of β-galactosidase expressed from a *RAD1-lacZ* fusion gene is not increased in cells exposed to DNA damage (177, 228). However, the possible induction of enhanced levels of *RAD1* mRNA or Rad1 protein has not been directly excluded. There is also no indication that this gene is cell cycle regulated (177). Disruption or deletion of the *RAD1* gene is not lethal to haploid yeast cells. Hence, *RAD1* is not an essential yeast gene (292). It has not been determined whether specific domains of the gene are required for nucleotide excision repair of DNA. However, deletions that include the first 11 codons of the *RAD1* ORF only partially inactivate the ability of the gene to complement the phenotype of *rad1* cells (89).

Examination of a hydrophilicity plot of the translated *RAD1* nucleotide sequence shows the presence of hydrophilic domains at the amino- and carboxy-terminal regions of the putative polypeptide (Fig. 3). The presence of hydrophilic carboxy- or amino-terminal domains or both is characteristic of several of the translated Rad polypeptide sequences (see below).

An intriguing clue concerning one of the possible functions of the *RAD1* gene has emerged from recent studies on transcriptionally linked genetic recombination in *S. cerevisiae*. Keil and Roeder (117) discovered a recombinational hot spot in the yeast genome designated *HOT1*. *HOT1* is part of the ribosomal DNA repeat unit and stimulates recombination by a transcription-dependent mechanism (268). Of the small number of yeast mutants thus far tested, only *rad1* mutations result in a reduction of *HOT1*-stimulated recombination (R. Keil, personal communication). This observation leads to the interesting possibility that Rad1 protein might be involved in the unfolding of the genome during transcription, thereby facilitating transcription-dependent recombination (and possibly repair).

The *RAD1* gene has been tailored into expression vectors for overexpression of Rad1 protein in *E. coli* (A. J. Cooper and E. C. Friedberg, unpublished observations) and in yeast (Yang, Ph.D thesis). Transcription of the cloned gene in *E. coli* can be regulated from the *tac* promoter with the lactose analog isopropylthiogalactoside. Overexpression in *E. coli* yields a protein which migrates in denaturing polyacrylamide gels with a calculated M_r of ~150,000, significantly higher than that (~126,000) calculated from the coding sequence of the cloned gene (Table 2) (Cooper and Friedberg, unpublished observations).

Overexpression in *S. cerevisiae* from the regulatable *GAL1* promoter results in a 350-fold enhancement of *RAD1* mRNA (Yang, Ph.D thesis). Under these conditions, Rad1 protein can be detected in crude extracts by immunoblotting, using affinity-purified rabbit antisera raised against the protein expressed in *E. coli* (Cooper and Friedberg, unpublished observations). Some of the Rad1 protein overexpressed in *S. cerevisiae* is soluble in aqueous solvents. However, much of the protein detected by immunoblotting can only be recovered by extraction of cell pellets with non-ionic detergents. Both the soluble and the insoluble proteins overexpressed in yeast cells also migrate in sodium dodecyl sulfate-polyacrylamide gels with an apparent M_r of ~150,000 (Table 2) (Cooper and Friedberg, unpublished observations). In this regard, it is relevant to note that Rad1 protein is expected to have an acidic pI, since its calculated net charge at pH 7.0 is −36 (Table 2), and that anomalous electrophoretic mobility has been reported with other acidic proteins (30, 102).

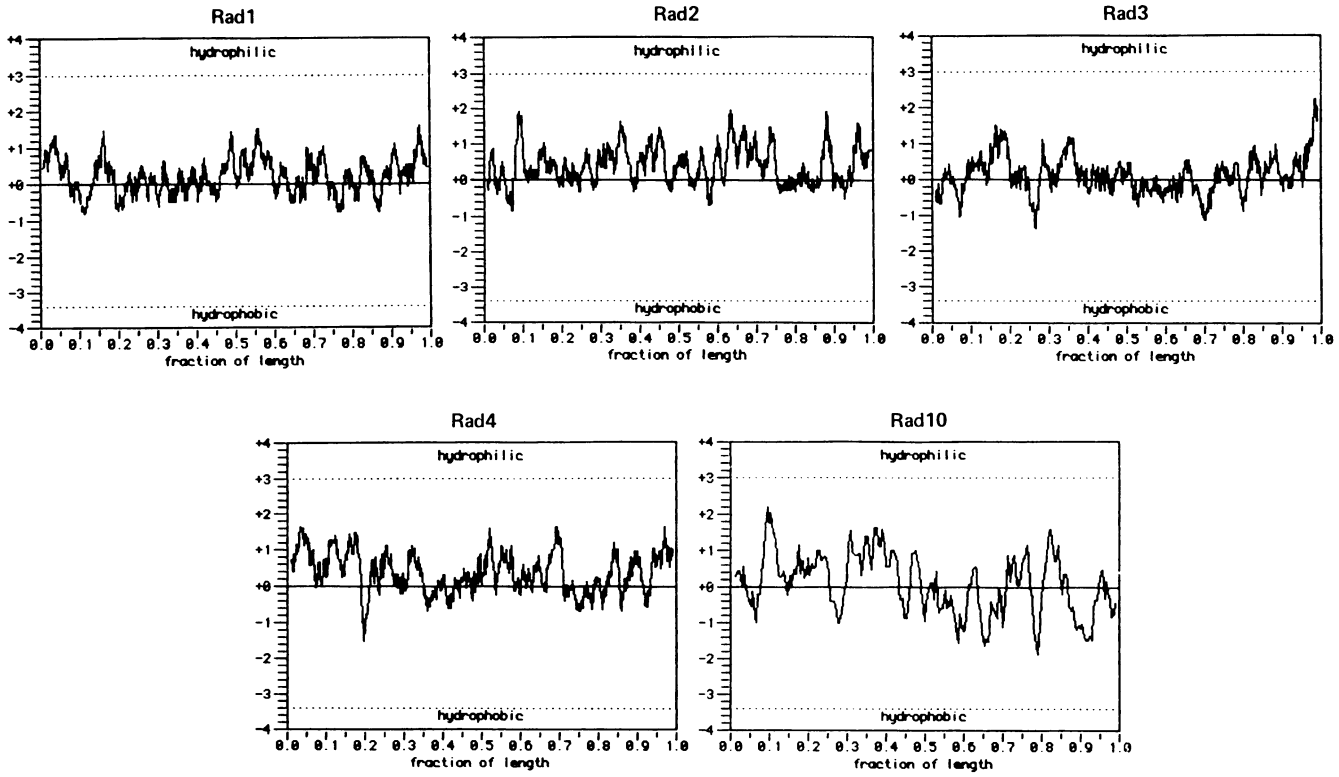

FIG. 3. Hydrophilicity plots of the predicted polypeptides translated from the nucleotide sequences of the cloned genes shown. The plots were derived by computer analysis, using the DNA Inspector II$^+$ program of Textco (which uses the Hopp and Woods analysis). The averaging lengths for the analyses were set at arbitrary intervals between 6 and 18 amino acids (depending on the length of the polypeptide) in the interests of clearer visual presentation.

***RAD2* gene.** The cloned *RAD2* gene (87, 182) contains an ORF of 3,093 base pairs (bp) (150) and is expected to encode a protein of calculated M_r 117,700 (Table 2). Like *RAD1*, the *RAD2* gene does not show extensive sequence similarities to other cloned genes, including the *E. coli uvr* genes, the human *ERCC1* gene, and other yeast *RAD* genes for which sequence information is available (150, 185). The gene encodes a transcript of ~3.2 kb which is weakly expressed in constitutive cells (steady-state levels, <five copies per cell [182]). As is true of many yeast transcripts, the 5′ ends of those encoded by the *RAD2* gene show considerable heterogeneity and map to sites located 9 to 60 nucleotides upstream of the translational initiation codon (185). A major transcriptional start site is located ~20 nucleotides upstream of the first coding ATG (185). *RAD2-lacZ* fusions express low levels of β-galactosidase activity (56). Hence, like *RAD1*, the *RAD2* gene of *S. cerevisiae* is a weakly expressed gene. Consistent with this observation, the *RAD2* coding region shows no bias against rare codons (150, 185).

In contradistinction to the *RAD1*, *RAD3*, *RAD4*, and *RAD10* genes, the *RAD2* gene is inducible by treatment of cells with DNA-damaging agents (150, 228). This can be demonstrated by transforming cells with plasmids containing *RAD2-lacZ* fusions and measuring the level of β-galactosidase after exposure to DNA damage. Such experiments show that treatment of cells with UV radiation, ionizing radiation, 4-nitroquinoline-1-oxide, or nalidixic acid increases the level of β-galactosidase three- to fivefold. Direct measurement of the steady-state level of *RAD2* mRNA shows a parallel increase (150, 228). Altered levels and kinetics of induction were also observed following treatment of cells with bleomycin, nitrogen mustard, mitomycin C, MMS, or methotrexate (228). Heat shock did not effect induction of *RAD2* (228). Consistent with a requirement for new protein synthesis, enhanced expression of *RAD2* is inhibited in the presence of cyclohexamide (228).

The mechanism of inducible regulation of the *RAD2* gene is not yet understood in detail. It has been determined that levels of *RAD2* mRNA do not vary during the cell cycle (S. Prakash, personal communication). This is an important consideration in establishing whether DNA-damaging agents are direct inducers of a gene or whether apparent induction results from arrest of cells at a particular stage of the cell cycle (128, 291), during which there may be enhanced transcription of the gene in question due to cell cycle regulation. Such cell cycle effects following DNA damage have been suggested as an explanation for induction of the *RAD6* gene (see later).

Regardless of the specific role of DNA-damaging agents, deletion analysis of the 5′ noncoding region of *RAD2* suggests that the gene is positively regulated. Thus, some deletions examined eliminate enhanced expression of β-galactosidase from *RAD2-lacZ* fusions following exposure of transformed cells to UV radiation, as might be expected if they removed sites at which one or more stimulatory proteins bind. No deletions thus far studied result in spontaneous induction, as might be expected if they removed sites for binding of repressors, i.e., if the gene was negatively regulated (G. W. Robinson, D. Kalainov, and E. C. Friedberg, unpublished observations).

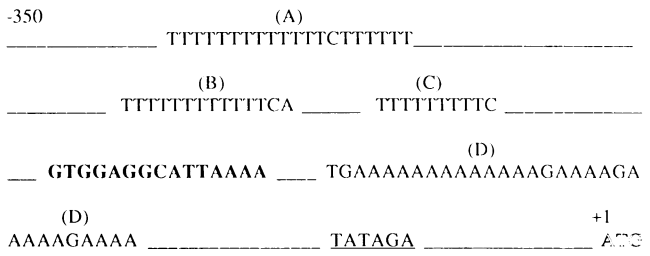

FIG. 4. Diagrammatic representation of sequences in the 350-bp region upstream of the *RAD2* coding region. The spacings between the sequences are not drawn to scale. (A) to (D) represent a series of AT-rich tracts that flank a putative UAS (bold type). A possible TATAAA box is shown downstream of the UAS and is underlined for identification. The ATG translational start codon at nucleotide position +1 is also shown.

Deletion mapping has identified a region located ~154 to 166 nucleotides upstream of the translational start codon which might function as an upstream activator sequence (UAS) for induction of *RAD2* (Fig. 4). In addition, deletions which include a number of poly(AT) tracts in the 5′ noncoding region (Fig. 4) inactivate the induction of *RAD2* (Robinson et al., unpublished observations). AT tracts have been implicated in the regulation of constitutive expression of a number of yeast genes (257). However, an alternative possibility for their role in *RAD2* expression is suggested by the observation that they flank the putative UAS (Fig. 4), and examination of their sequence suggests the potential for forming several stem-loop structures. Thus, induction of *RAD2* may require both binding of a regulatory protein to a UAS and conformational alterations which isolate the UAS in a looped structure. Clearly, further analysis of the *RAD2* upstream region is required to define the role(s) of those AT tracts more precisely.

The functional significance of this inducible response to DNA damage is not known. In view of the observation that other *RAD* genes (notably *RAD1* [228] and *RAD10* [G. W. Robinson, W. A. Weiss, and E. C. Friedberg, unpublished observations]) are also very weakly expressed in constitutive cells, it is not obvious why *RAD2* uniquely manifests this response to DNA damage. It might be informative to evaluate various biological and biochemical endpoints in cells in which the *RAD2* gene is induced and also in cells in which induction is crippled by mutational alteration of selected regulatory sites in the gene.

Translation of the *RAD2* coding region indicates that, like Rad1 protein, Rad2 protein is highly acidic. The calculated net charge at pH 7.0 is −35 (Table 2), and the polypeptide contains 17.8% acidic amino acid residues. Also, a hydropathicity plot of the translated *RAD2* sequence indicates that Rad2 protein is the most hydrophilic of the five Rad proteins required for incision of damaged DNA in *S. cerevisiae* (Fig. 3) and shows no pronounced hydrophobic regions. Like the Rad1 polypeptide, Rad2 contains a hydrophilic carboxy-terminal domain (Fig. 3). The carboxy-terminal tail of the translated sequence is very rich in basic amino acids; 18 of the last 41 amino acids are either Arg or Lys (Fig. 5). This highly basic domain apparently has functional significance (perhaps in binding to DNA or to other proteins), since deletion of the terminal 78 codons of the *RAD2* coding region inactivates the ability of the cloned gene to complement the UV sensitivity of *rad2* mutants (87).

Like Rad1 protein, Rad2 protein overexpressed in *E. coli* or in *S. cerevisiae* migrates in sodium dodecyl sulfate-polyacrylamide gels with an apparent molecular weight considerably larger (~150,000) than that predicted from the size of the coding region (186) (Table 2). This anomalous electrophoretic mobility is not an exclusive property of the protein overexpressed from the cloned gene, since immunoblotting of untransformed cells reveals the same property in endogenous Rad2 protein (186). The yeast protein is not phosphorylated and shows no evidence of glycosylation detectable by binding of concanavalin A (186). Hence, as suggested for Rad1 protein (see above), the anomalous electrophoretic mobility of the protein may be related to its highly acidic character. Much of the overexpressed Rad2 protein in yeasts is present in an insoluble form extractable with 1% Sarkosyl. However, a significant fraction of the protein is soluble to aqueous extraction and can be readily detected by immunoblotting with polyclonal antisera raised against native Rad2 proteins or Rad2/β-galactosidase fusion protein expressed in *E. coli* (185; H. Burtscher and E. C. Friedberg, unpublished observations).

***RAD3* gene.** The cloned *RAD3* gene (88, 180) contains a coding region 2,334 bp long (179, 219) and is expected to encode a polypeptide of calculated M_r 89,700 (Table 2). Overexpression of *RAD3* in *E. coli* and in yeast cells reveals a polypeptide of the expected size (L. Naumovski and E. C. Friedberg, Mol. Gen. Genet., in press; S. Prakash, personal communication). Hence, unlike Rad1 and Rad2 proteins, Rad3 protein does not show anomalous electrophoretic mobility. The amino acid sequence of the translated Rad3 polypeptide shows a hydrophilic tail in which 12 of the carboxy-terminal 20 amino acids are the acidic residues Glu and Asp (Fig. 5). Nonetheless, deletion of a region of the *RAD3* gene which encodes the terminal 78 amino acids has no detectable effect on *RAD3* functions (see later). Unlike Rad1 and Rad2 proteins, the Rad3 polypeptide shows several pronounced regions of hydrophobicity (Fig. 3).

The amino-terminal end of the putative Rad3 polypeptide contains a region which closely resembles a nucleotide-binding domain present in a number of other proteins, many of which are known adenosine triphosphatases (ATPases) (275). Of particular interest is a striking similarity between this region of the Rad3 polypeptide and regions in the amino acid sequence of the *E. coli* nucleotide excision repair proteins UvrA (100), UvrD (49), and UvrB (4, 9) and the recombinational repair proteins RecA (96, 238), RecC (51), and RecD (50) (Fig. 6). UvrA, UvrD, and RecA proteins have been shown to be ATPases (1, 246; see reference 55). Also, mixing UvrA protein with UvrB protein in vitro results in a marked increase in ATPase activity of the protein complex (188, 263), suggesting that this interaction may

FIG. 5. The carboxy-terminal 21 to 23 amino acids of the putative Rad2, Rad3, Rad4, and Rad6 polypeptides. Each has a high proportion of acidic (D or E) or basic (K or R) amino acids, especially Rad6. Amino acids are designated by the one-letter code.

UvrA	24-45	D	K	L	I	V	V	T	G	L	S	G	S	G	K	S	S	L	S	F	D	T	L
UvrA	633-654	G	L	F	T	C	I	T	G	V	S	G	S	G	K	S	T	L	I	N	D	T	L
UvrB	32-53	L	A	H	Q	T	L	L	G	V	T	G	S	G	K	T	F	T	I	A	N	V	I
UvrD	22-43	R	S	N	L	L	V	L	A	G	A	G	S	G	K	T	R	V	L	V	H	R	I
RecA	59-80	G	R	I	V	E	I	Y	G	P	E	S	S	G	K	T	T	L	T	L	Q	V	I
RecB	16-37	Q	G	E	R	L	I	E	A	S	A	G	T	G	K	T	F	T	I	A	A	L	Y
RecD	164-185	R	R	I	S	V	I	S	G	G	P	G	T	G	K	T	T	T	V	A	K	L	L
Rad3	35-56	G	G	N	S	I	L	E	M	P	S	G	T	G	K	T	V	S	L	L	S	L	T

FIG. 6. A consensus nucleotide-binding box (275) is present in the *E. coli* DNA repair proteins shown, all of which have also been shown to have ATPase activity under different experimental conditions. The UvrA polypeptide has two such sequences. The amino-terminal region of the Rad3 polypeptide shows a very good match to the boxed regions and led to the early prediction that it might also catalyze the hydrolysis of adenosine 5'-triphosphate.

unmask a cryptic activity in UvrB protein or directly stimulate the ATPase activity of UvrA protein. Evidence in support of cryptic activity associated with UvrB protein comes from recent studies by Grossman and his colleagues, who have shown that a form of UvrB protein derived by specific proteolysis in vivo (designated UvrB*) has strong ATPase activity (cited in reference 58).

It has been suggested that the *RAD3* and *E. coli uvrD* genes share amino acid sequence homology with a yeast nuclear gene designated *PIF1* (54). This gene is required for both recombination repair of mitochondrial DNA and recognition of a recombinogenic signal characterized by a 26-bp palindromic AT sequence in the *ery* region of mitochondrial DNA (54). Mutants defective in *PIF1* show a dramatic increase in the fraction of cytoplasmic petite mutations (*rho*$^-$ cells) after UV irradiation or treatment with ethidium bromide. The cloned *PIF1* gene contains an ORF which could encode a protein of calculated M_r 97,500, a value very close to that of Rad3 protein (see below). Like *RAD3* (see below), the translated amino acid sequence of *PIF1* shows a nucleotide-binding domain near the amino-terminal end and a potential specific DNA-binding (helix-turn-helix) domain near the carboxy-terminal end (54).

Rad3 protein can be overexpressed in *E. coli* and in yeast cells. In the former case, the majority of the protein is associated with an insoluble fraction of cell extracts (Naumovski and Friedberg, unpublished observations). When overexpressed in yeast cells, however, much of the Rad3 protein can be isolated in the aqueous fraction of cell extracts (L. Naumovski and E. C. Friedberg, in press). Not unexpectedly, Rad3 protein expressed from the single-copy chromosomal gene in untransformed yeast cells, as well as that overexpressed in transformed cells, is located in the nucleus (Naumovski and Friedberg, in press).

Rad3 protein has been purified to apparent homogeneity from cells transformed with a multicopy plasmid carrying the cloned gene under control of the *ADC1* promoter (260). The purified protein has ATPase activity that is strictly dependent on the presence of single-stranded DNA or polydeoxyadenylate and is partially inhibited by antiserum containing rabbit antibodies to a truncated Rad3 protein expressed in *E. coli* (260). Experiments in my laboratory have independently confirmed these findings, using both partially purified native Rad3 protein and a Rad3/β-galactosidase fusion protein extensively purified by affinity chromatography by using an immobilized substrate for β-galactosidase (Naumovski and Friedberg, in press). This ATPase activity has not been characterized in detail, and properties that might distinguish it from previously characterized ATPases of *S. cerevisiae* are not yet determined.

It has been independently demonstrated that extracts of *rad3* mutant strains have little or no detectable ATPase III activity (259). (ATPase III is a DNA-dependent ATPase with associated DNA helicase activity and also stimulates DNA synthesis in an in vitro assay system [259]). Several lines of evidence suggest that the putative ATPase encoded by the *RAD3* gene and ATPase III of *S. cerevisiae* are distinct proteins. The latter enzyme has an M_r of ~63,000 (259). Furthermore, antibodies against Rad3 protein do not react with ATPase III in Western blots (immunoblots) (259). These results do not eliminate the possibility that the 90-kilodalton (kDa) Rad3 polypeptide contains cryptic ATPase III activity which can be activated by proteolytic processing to a 63-kDa form of the protein and that the failure of Rad3 antibodies to cross-react with ATPase III reflects the loss of strong epitopes in a domain which is removed during this processing event. However, this is an unlikely explanation for the failure to observe an immune reaction between Rad3 antibodies and ATPase III, since several truncated forms of Rad3 protein have been shown to react strongly with antisera raised against full-length Rad3 protein (Naumovski and Friedberg, in press).

The reason for the failure to detect ATPase III activity in extracts of *rad3* mutants is unclear. However, several possibilities merit consideration. First, as is the case with the UvrAB complex of *E. coli* (188, 263), the interaction of Rad3 protein with ATPase III may normally stimulate ATPase activity in the complex, and the failure to detect ATPase III activity in extracts of *rad3* mutants may reflect a lack of stimulation by mutant Rad3 protein. A physical association of Rad3 and ATPase III proteins might also influence the affinity of the latter for chromatographic matrices used for its purification. Hence, ATPase III might fractionate differently from extracts of *rad3* mutants relative to *RAD3* cells, leading to the erroneous impression that it is defective in the former cells. Finally, Rad3 protein may be required to activate ATPase III from an inactive precursor, or it may even be required to activate the gene that encodes the ATPase. Regardless of the precise relationship between the putative ATPase activity of Rad3 and ATPase III of *S. cerevisiae*, there is good evidence that these two ATPases represent distinct polypeptides. I therefore suggest that the ATPase activity of Rad3 protein be designated ATPase IV of *S. cerevisiae*.

The observation that ATPase III activity is diminished in extracts of cells defective in the excision repair function of the *RAD3* gene (259) suggests that the gene which encodes ATPase III may also be involved in nucleotide excision repair in yeasts. Of the genes in the *RAD3* epistasis group thus far cloned and sequenced, the only one with a coding region that approximates the size of purified ATPase III is *RAD7* (196). This gene could encode a protein of calculated M_r 63,700 (see later). However, there is no evidence for a defect in ATPase III in extracts of *rad7* mutant cells (A. Sugino, unpublished observations). The *RAD14*, *RAD16*, *RAD23*, and *MMS19* genes are also possible candidates for the ATPase III gene. However, these genes have not yet been isolated by molecular cloning and the sizes of their coding regions are not known. The availability of purified ATPase III might facilitate the molecular cloning of the gene,

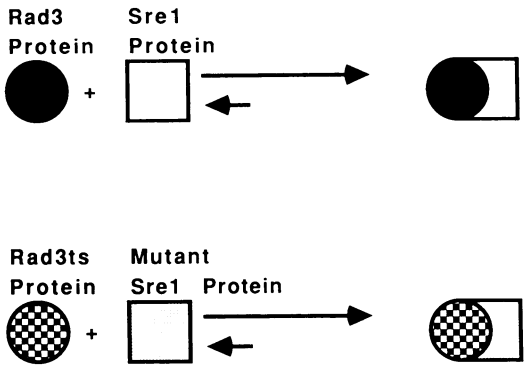

FIG. 7. Model for suppression of a mutation in the *RAD3* essential function by expression of normal levels of a mutant *SRE* gene. This model is derived from that of Jarvik and Botstein (106). It proposes that mutations in genes which encode products that normally interact with wild-type Rad3 protein can compensate for the defective protein-protein interaction and hence suppress the mutant phenotype.

using antibody or oligonucleotide probes derived from the sequence of the purified protein.

An analogous phenomenon has been observed with a different enzyme activity in extracts of *rad52* mutants. These extracts are defective in a yeast nuclease activity that cross-reacts with antibodies raised against a *Neurospora crassa* nuclease believed to be involved in recombination and repair in that organism (34, 214, 218). However, the molecular weight of the purified yeast enzyme suggests that it is encoded by a gene other than *RAD52* (214), and very recently the gene which encodes this nuclease was isolated by molecular cloning and shown to be distinct from the *RAD52* gene (T. Chow, personal communication) (see later discussion).

Random mutagenesis of the *RAD3* coding region indicates that single missense mutations scattered throughout the gene can inactivate the excision repair function of this gene (183). The marked sensitivity of Rad3 protein to inactivation by many different point mutations provides a rational explanation for the isolation of a large number of excision-defective *rad3* mutants. Site-specific mutagenesis targeted to the putative nucleotide-binding domain also inactivates the *RAD3* excision repair activity (183; S. Prakash, personal communication). However, in view of the fascile inactivation of the DNA repair function, these results are consistent with but not proof of the functional significance of this domain.

Disruption of the chromosomal *RAD3* gene is lethal to haploid yeast cells and confers recessive lethality to diploid cells (88, 181). None of the other four *RAD* genes required for nucleotide excision repair confer this phenotype when mutated (52, 182, 280, 292). It has recently been shown that a gene designated *RAD24* (44) (formerly called r_1^- [6]), which is classified in both the *RAD3* and *RAD50* epistasis groups (44) (Table 1) and which genetically maps very close to *RAD3* (171), is also an essential gene. Unconditional single missense mutations widely distributed throughout the *RAD3* coding region do not inactivate the essential function of the gene (183). However, a mutant bearing tandom missense mutations at codon positions 595 and 596 near the 3′ end of the gene is inviable (183). It has not been definitively established that this region of *RAD3* represents a specific domain required for the essential function. Nonetheless, it is interesting that codons 595 and 596 are situated in a region of the gene in which the translated amino acid sequence resembles that of the helix-turn-helix motif that characterizes many specific DNA-binding proteins from both procaryotes and eucaryotes (183).

The nature of the *RAD3* essential function is not known. The observation that mutants completely defective in excision repair of DNA are viable suggests that the repair and essential functions of the gene are distinct. However, it is difficult to rule out the possibility that the same structural or catalytic activity is subserved by both functions, but the excision repair function is more sensitive to a reduction in the amount of active protein or to mutations which alter the conformation of the protein.

An obvious approach to investigating the *RAD3* essential function is to isolate mutants with conditional lethal mutations in this gene. A single temperature-sensitive mutant designated *rad3-1*(Ts) was recently isolated (184), using a protocol originally described by Shortle et al. (248). Following a shift to nonpermissive temperatures, this mutant can only complete two to four cell doublings before growth ceases. The property of limited growth at restrictive temperatures may reflect leakiness of the *rad3-1*(Ts) allele. On the other hand, following disruption of the *RAD3* gene in diploid cells, haploid spores which inherit the disrupted gene also manifest limited growth and give rise to abortive colonies (181). A similar phenotype has been noted for several other essential yeast genes, including *RAD24* (J. Game, personal communication) and following disruption of a gene called *RPC40*, which encodes a subunit of yeast RNA polymerase III (156). This "leaky" phenotype may reflect the fact that many essential proteins are components of multiprotein complexes. A shift to nonpermissive conditions may inactivate nascent proteins and hence preclude the assembly of new functional complexes. However, complexes formed prior to the temperature shift may retain functional integrity and provide cells with a limited capacity for normal metabolism under restrictive conditions.

A detailed study of the *rad3-1*(Ts) mutant has not provided an explanation for the *RAD3* essential function. The relative kinetics of inhibition of DNA, RNA, and protein syntheses at nonpermissive temperatures are approximately the same; hence, there is no evidence that any of these parameters of cellular metabolism are preferentially inactivated (184). Based on the premise that Rad3 protein normally interacts with other proteins (some of which may also be essential), my colleagues and I have recently initiated a search for genes which suppress the phenotype of conditional lethality imparted by the *rad3-1*(Ts) mutation. Suppressors can in theory be identified by at least two experimental approaches. One is to identify mutant genes which complement the mutation in the *RAD3* gene. Such extragenic suppressors (106, 116) might be expected to encode proteins which, when conformationally altered, are able to interact with conformationally altered Rad3 protein in a manner that restores activity to the latter protein (Fig. 7). Such an experimental approach was extremely useful in elucidating the biological role of the yeast *RAS* genes in the metabolism of cyclic adenosine 5′-monophosphate (115). An alternative approach is to identify wild-type genes, which, when overexpressed, complement the defective protein of interest. Such suppressors may compensate for a lowered binding affinity of the mutated protein for other proteins with which it normally interacts (Fig. 8).

In the case of *rad3-1*(Ts), two suppressor genes in the latter category were isolated by screening a yeast genomic library of multicopy plasmids with wild-type recombinant

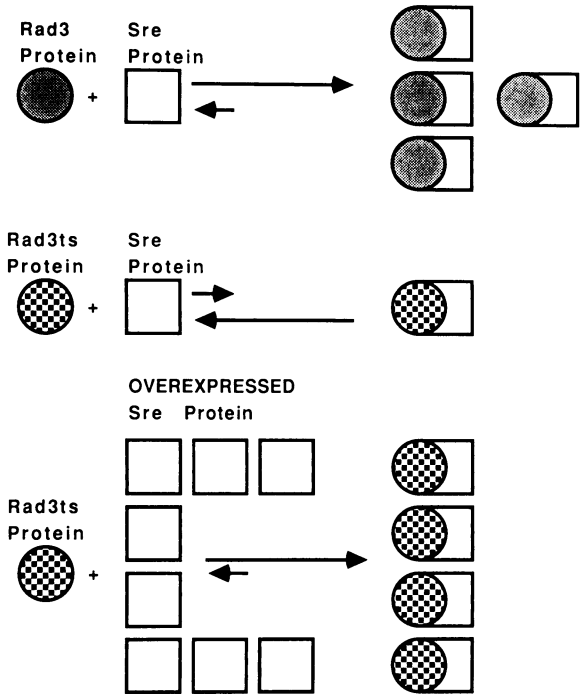

FIG. 8. Model for phenotypic suppression of a mutation in the *RAD3* essential function by overexpression of a wild-type gene, *SRE* (suppressor of RAD3 essential function). The model predicts that the interaction of Rad3 and Sre proteins normally results in a high-affinity complex necessary for the Rad3 essential function. The mutant protein Rad3(Ts) binds weakly to Sre protein. However, when Sre protein is overexpressed, enough stable Rad3/Sre complex is made to support the essential function.

inserts (184). One of the genes (designated *SRE1* [suppressor of RAD3 essential function]) partially suppresses the lethality of the *rad3-1*(Ts) mutant at 37°C. Disruption analysis has shown that, unlike *RAD3*, *SRE1* is not an essential yeast gene. However, there are indications that the gene does not act nonspecifically. For example, suppression is diminished when the gene is transformed into cells on a single-copy plasmid. Also, the *SRE1* gene does not suppress conditional lethal mutations in a number of other essential yeast genes tested. It also does not suppress the phenotype of *rad3* disruption mutants; i.e., in the absence of Rad3 protein *SRE1* does not simply bypass *RAD3* function (184).

A second suppressor gene (designated *SRE2*) suppresses the phenotype of the *rad3-1*(Ts) more weakly. This gene also has no detectable suppressor activity when present on a single-copy plasmid. Disruption of *SRE2* has not yet been carried out; hence, it is not known whether it is an essential gene. A partial sequence of the *SRE1* gene revealed an ORF of 180 amino acids. A computer-assisted search demonstrated a region of similarity with two glycolytic enzymes, glyceraldehyde 3-phosphate dehydrogenase from *Bacillus subtilis* and phosphofructokinase from rabbit muscle (R. F. Doolittle, personal communication) (Fig. 9). These similarities are of possible evolutionary interest and may indicate conservation of structural domains in the three polypeptides. However, it seems improbable that the *SRE1* or *RAD3* gene is involved in glycolysis in yeast cells.

Certain mutations in *RAD3* (designated *rem*) confer the phenotype of increased spontaneous mutation and increased spontaneous mitotic recombination. The *rem1-1* allele was originally isolated by Golin and Esposito (71). More recently, a second allele (*rem1-2*) was isolated and characterized by Malone and Hoekstra (155). The *rem* mutants are not sensitive to UV radiation and hence are presumably not defective in nucleotide excision repair. However, detailed characterization of the excision repair phenotype of these mutants has not been reported. The *rem1 rad52* and *rem1 rad50* double mutants are inviable, and this observation has led to the suggestion (94) that *rem* strains contain recombinogenic lesions (possibly double-strand breaks), which are lethal unless repaired by the recombinational repair pathway represented by the *RAD50* epistasis group of genes. More recently, it has been reported that this lethality can be rescued by a third mutation affecting genes (other than *RAD3*) in the nucleotide excision repair pathway (B. A. Montelone, M. F. Hoekstra, and R. E. Malone, personal communication). This suggests that, to be lethal, lesions generated in the DNA of *rem1* mutants must be acted on by products of excision repair genes. Montelone et al. (personal communication) speculate that the lesions in *rem1* cells may be nucleotide mismatches introduced into the genome as DNA replication errors. They have proposed that in *rem* mutants these mismatches may be processed by cellular components that include nucleotide excision repair proteins, thereby generating double-strand breaks repairable by the *RAD50* group of genes.

rad3-1(Ts) *rad50* double mutants are viable at nonrestrictive temperatures and do not show evidence of enhanced lethality at intermediate temperatures or at 37°C (184). Furthermore, the conditional lethality of *rad3-1*(Ts) cannot be rescued by mutations in other genes involved in nucleotide excision repair. Hence, it is unlikely that the *rad3-1*(Ts) mutation is defective in Rem functions.

A final interesting and as yet unexplained phenotype of *rad3* mutants warrants brief description. It has been shown that a number of yeast genes other than *RAD2* are inducible by treatment of cells with DNA-damaging agents (35, 47, 110, 157, 197, 232, 233). One of these genes, designated *DDRA2*, shows a significantly reduced level of induction of mRNA in a *rad3-2* mutant following treatment of cells with the UV-mimetic chemical 4-nitroquinoline-1-oxide or with the alkylating agent MMS (151). In addition, the kinetics of induction of a second inducible gene (*DDR48*) are altered in *rad3* mutants relative to *RAD3* cells (151). It remains to be established whether these effects are specific to *RAD3* or

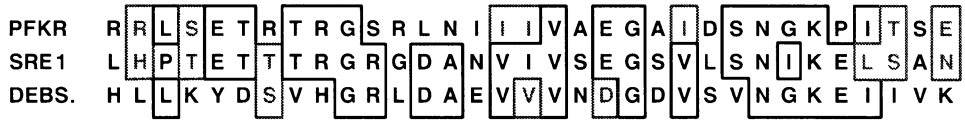

FIG. 9. Amino acid sequence similarities between regions of rabbit muscle phosphofructokinase (PKFR), the yeast *SRE1* gene, and glyceraldehyde 3-phosphate dehydrogenase of *B. subtilis* (DEBS). Identical amino acids (one-letter code) are in black boxes; related amino acids are in grey boxes.

FIG. 10. Amino acid sequence of putative Rad4 and Rad10 polypeptides showing similarities over a limited domain. Identical amino acids (one-letter code) are boxed with solid lines; related amino acids are boxed with dashed lines.

reflect a more general requirement for all excision repair functions.

***RAD4* gene.** The complete nucleotide sequence of the *RAD4* gene has recently been determined (L. Couto and E. C. Friedberg, unpublished observations). The coding region consists of 2,262 bp and could encode a protein with a calculated molecular weight of ~87,100. The putative Rad4 polypeptide has a net charge of +4 at pH 7. Examination of a hydrophilicity plot of the putative Rad4 polypeptide shows hydrophilic amino- and carboxy-terminal domains (Fig. 3). Also, as is the case in the Rad3 (see above) and Rad6 (see later) polypeptides, the terminus of the Rad4 polypeptide contains a large fraction of Glu and Asp residues (11 of the last 22 amino acids) (Fig. 5). *RAD4* shows no codon bias and is expected to be a weakly expressed yeast gene. Preliminary searches of available DNA and protein data bases showed no extensive similarity with other genes or polypeptides, including the other sequenced *RAD* genes. Interestingly, however, a comparison of the region of the Rad4 polypeptide between amino acids 111 and 134 with that between amino acids 33 and 55 in the Rad10 polypeptide shows that 10 of the 24 amino acids are identical and a further 6 are related (Fig. 10). Since Rad4 and Rad10 (see later) are the only two positively charged proteins of the six Rad proteins from the *RAD3* epistasis group under consideration (Table 2), this conserved domain may have functional or structural relevance.

As indicated earlier, transformation of *E. coli* with plasmids containing the wild-type *RAD4* gene results in mutational inactivation, as evidenced by undetectable or weak phenotypic complementation of UV sensitivity when single-copy (centromeric) plasmids containing mutationally altered *RAD4* alleles are transformed into *rad4* mutants (52). More recently, it has been demonstrated that when some of these mutant alleles are present on multicopy plasmids, they can result in considerable phenotypic complementation, indicating that the proteins encoded by these mutated genes can retain functional activity for nucleotide excision repair when overexpressed (53). The sites of mutational inactivation in some of these *RAD4* alleles have been determined by DNA sequencing (Couto and Friedberg, unpublished observations). These mutations are expected to result in truncated Rad4 polypeptides in which as much as half of the protein is missing, suggesting that Rad4 function is remarkably insensitive to extensive alterations of the protein. Rad4 is not an essential yeast gene (52), and the steady-state levels of *RAD4* mRNA are not increased in cells exposed to DNA-damaging agents (52).

***RAD10* gene.** The *RAD10* gene is the smallest of the five genes required for excision repair of UV-irradiated DNA. The coding region is only 630 bp long and could encode a polypeptide of calculated M_r ~24,300 (220). Rad10 protein contains several hydrophobic domains (Fig. 3); in fact, much of the carboxy-terminal half of the protein is hydrophobic in nature. The amino- and carboxy-terminal regions of the polypeptide are not unusually charged (Fig. 3). The calculated net charge of the polypeptide at pH 7 is +3. The distribution of charged residues along the Rad10 polypeptide chain is not random. In particular, it has been noted that amino acids 78 to 159 contain 3 acidic and 17 basic residues and that 8 of the 10 Tyr residues in the protein are clustered in this region (220). A clustering of Tyr residues has been noted in several procaryotic DNA-binding proteins, including gene 5 protein from phage fd (3) and gene 32 protein of phage T4 (163). It has been independently suggested (266) that the region between amino acids 110 and 140 is a reasonable match for the consensus helix-turn-helix motif that characterizes specific DNA-binding proteins and which was referred to above in the context of *RAD3*. The *RAD10* gene is not essential and disruption of the gene has no effect on the viability of haploid cells (280). There is also no evidence that expression of *RAD10* is enhanced in cells exposed to DNA-damaging agents (Robinson et al., unpublished observations).

The translated amino acid sequence of the *RAD10* gene shows extensive similarity to that of a putative human DNA repair gene designated *ERCC1* (266). The complementary DNA for this gene was isolated by phenotypic complementation of the UV sensitivity of a Chinese hamster mutant cell line defective in nucleotide excision repair (266). The *ERCC1* gene (human excision repair gene which complements Chinese hamster ovary cells) is at least 18 kb, but the size of the complementary DNA is close to that of the *RAD10* gene. The sequence similarity between *RAD10* and *ERCC1* provides persuasive evidence that the latter is indeed a DNA repair gene and suggests that eucaryotic DNA repair genes may be generally conserved. The issue of structural and functional conservation of excision repair functions is discussed more fully later.

Rad10 protein has been overexpressed in yeasts and is detected at an M_r of 24,000, as expected from the size of the coding region. Much if not all of the Rad10 protein overexpressed in yeast cells is soluble in aqueous solvents and should be amenable to purification by conventional fractionation techniques (W. A. Weiss and E. C. Friedberg, unpublished observations).

Other Cloned Yeast Genes from the *RAD3* Epistasis Group

***RAD7* gene.** The *RAD7* gene is one of at least seven tightly linked genes that are clustered on the right arm of chromosome X (162). This region of the yeast genome (representing about 38 kb of DNA) was isolated by chromosome walking and divided into 13 fragments which were cloned into yeast plasmid vectors (196). A plasmid containing a fragment that includes *RAD7* was identified by phenotypic complementation of a *rad7* deletion mutant. The cloned gene contains a single ORF 1,695 bp long, which is expected to encode a protein of calculated M_r ~63,700. As indicated above, this is almost exactly the size of the purified ATPase III protein described in relation to the *RAD3* gene.

Like the other *RAD* genes previously discussed, *RAD7* shows no codon bias. The only rare codon not used in this gene is CCC, encoding proline. Hence, it is likely that *RAD7* is also a weakly expressed gene. The translated amino acid

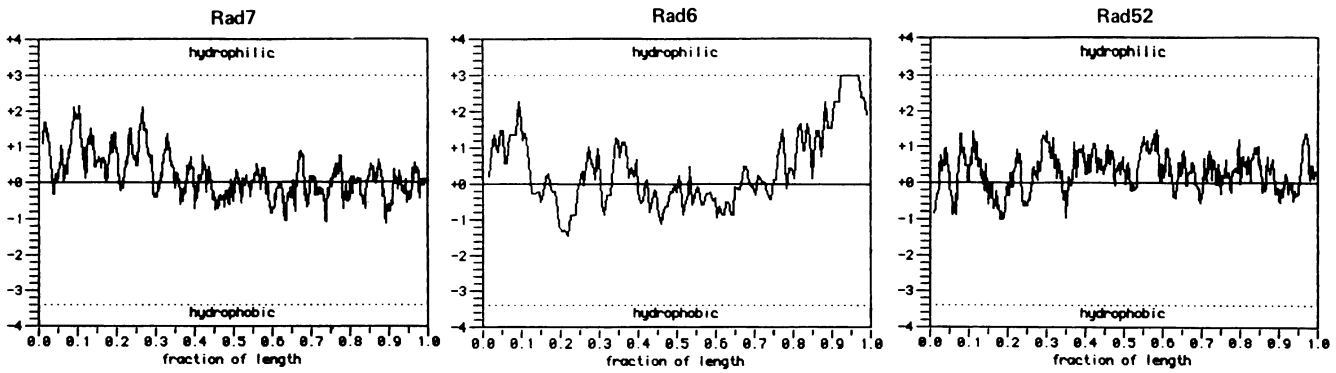

FIG. 11. Hydrophilicity plots of the predicted Rad7, Rad6, and Rad52 polypeptides. See legend to Fig. 3 for details.

sequence shows no homology with other proteins. The calculated net charge of Rad7 protein at pH 7.0 is +13. Most of the charged amino acids are concentrated towards the amino-terminal third of the polypeptide chain, and a hydrophilicity plot of the translated amino acid sequence indicates that this end of the polypeptide is very hydrophilic (Fig. 11). Deletion of the amino-terminal 99 amino acids did not affect the ability of multicopy plasmids to complement the UV sensitivity of the *rad7* deletion mutant (196). However, when this deleted gene was subcloned into a low-copy vector, complementation was only partial, and when the *rad7* mutant also carried a deletion in the *rad23* gene, no complementation of UV sensitivity was observed (196). Thus, while the hydrophilic amino terminus of Rad7 protein is apparently required for normal excision repair, the role of this protein can be subserved by Rad23 protein. This interpretation is consistent with evidence suggesting a close evolutionary relationship between regions of the yeast genome containing the *RAD7* and *RAD23* genes, since both genes are closely linked to two highly conserved cytochrome oxidase genes, *CYC1* in the case of *RAD7* and *CYC7* in the case of *RAD23* (162). These considerations notwithstanding, overexpression of Rad7 protein from a multicopy plasmid cannot complement the UV sensitivity of a *rad23* mutant and vice versa, suggesting that the two polypeptides are not mutually interchangeable for their function in excision repair.

***CDC8* gene.** The *CDC8* gene was originally defined as one of a number of genes required for normal cell cycle progression in yeasts (76). A temperature-sensitive *cdc8* mutant was shown to cease DNA synthesis when incubated at restrictive temperatures (77), suggesting that the cell cycle block in this mutant is the result of arrested DNA replication. Prakash et al. (203) observed decreased mutagenesis at a number of genetic loci when *cdc8* cells were exposed to UV radiation. In addition, the *cdc8* mutant was shown to be moderately UV sensitive at permissive temperatures. In view of the fact that many mutants with these phenotypes fall into the *RAD6* epistasis group (see later), these investigators studied the epistatic relationship of this mutation to a number of the *rad* mutations. By this analysis the *CDC8* gene was classified in both the *RAD6* and *RAD3* epistasis groups (203) (Table 1). The latter result suggests a role for this gene in nucleotide excision repair.

More recently, the *CDC8* gene was isolated by molecular cloning (18, 124) and was shown to be the structural gene for thymidylate kinase (112, 244). The cloned gene contains an ORF of 216 codons and could encode a polypeptide of calculated M_r 24,792, in good agreement with the measured molecular weight of the purified kinase (112).

The role (if any) of thymidylate kinase in excision repair of DNA is not known. Based on observations in procaryotes and mammalian cells (210, 211), it has been argued that enzymes involved in the biosynthesis of precursors for DNA replication may exist in cells as multiprotein complexes which are part of the replisome and which channel these precursors into the replication fork during semiconservative DNA replication. Conceivably, such enzymes are also components of a multiprotein "repairosome" in yeasts, and this model provides yet another possible explanation for the genetic complexity of nucleotide excision repair in this eucaryote.

***CDC9* gene.** Another conditional lethal mutant defective in DNA replication carries a mutation in a gene designated *CDC9*, also originally identified as a gene required for normal cell cycle events (78) and subsequently shown to be the structural gene for DNA ligase (10, 110). Not unexpectedly (based on the premise that all excision repair of DNA requires DNA ligation), preincubation of the temperature-sensitive mutant *cdc9-1* at nonpermissive temperatures followed by UV irradiation and incubation at permissive temperatures results in a significant increase in UV sensitivity of the mutant (109).

The *CDC9* gene was cloned by phenotypic complementation of a DNA ligase-defective mutant of *S. cerevisiae* (11). Mutants harboring several different *cdc9* alleles have been shown to encode temperature-sensitive forms of DNA ligase, thus confirming that the *CDC9* gene is the structural gene for this enzyme (10). The cloned gene contains an open reading frame 2,265 bp long and can encode a protein of calculated M_r ~84,800 (12). The translated nucleotide sequence shows limited overall similarity to DNA ligase from phage T7 or T4, the only other forms of DNA ligase for which sequence information is currently available. However, a stretch of ~45 amino acids shows considerable similarity between these three DNA ligases and RNA ligase from phage T4 and includes the lysine residue in T4 RNA ligase known to bind adenosine 5'-monophosphate (262). The cloned gene from *S. cerevisiae* is able to complement a DNA ligase-deficient mutant from the fission yeast *Schizosaccharomyces pombe* designated *cdc17* (11).

Enhanced expression of the *CDC9* gene has been observed both during the cell cycle and in response to DNA-damaging agents, and the important question arises as to whether these regulatory effects are related or distinct. Using synchronous cultures prepared by exposing cells to alpha factor, Peterson et al. (197) observed at least a fourfold increase in the steady-state level of *CDC9* mRNA in late G1, after the completion of the start phase of the cell cycle, but

before bud emergence and the onset of S phase. This cell cycle regulation of *CDC9* was shown to be independent of the *CDC7* and *CDC8* functions. These investigators also reported about a ninefold increase in the steady-state level of *CDC9* mRNA in cells exposed to either UV radiation or MMS (197).

Using three distinct cell synchronization methods, White et al. (285) confirmed the increase in *CDC9* transcripts in late G1 phase. In their hands, mRNA levels reached a peak at about the G1/S boundary and preceded the peak of maximal histone mRNA expression by about 20 min. Despite a significant increase in mRNA, the level of DNA ligase activity only increased about 50%, suggesting that the constitutive level of the enzyme is high and that the enzyme has a long half-life.

White et al. (285) also confirmed the induction of *CDC9* in response to UV radiation in mid-log-phase cells. As indicated earlier, evaluation of the apparent inducibility by DNA damage of genes known to be cell cycle regulated requires special attention, since DNA-damaging agents can cause partial synchronization of cell populations; hence, there is a potential for confusing cell cycle regulation with induction caused by DNA damage. Mindful of this pitfall, Johnson et al. (108) examined the response of cells to UV radiation in the stationary phase, when the level of *CDC9* mRNA was demonstrably low and is not cell cycle regulated. Under these conditions, a sharp increase of about ninefold was observed in the steady-state level of mRNA, suggesting that the gene is indeed induced by exposure of cells to DNA-damaging agents in a manner independent of the cell cycle. Once again, the level of DNA ligase activity was only increased about 50%, and in contrast to the decline in the basal level of *CDC9* transcripts in cells held in the stationary phase, the basal level of enzyme activity remained unaffected for up to 12 weeks. The enzyme is therefore very stable under all growth conditions, and these observations prompt the admittedly teleological question, What is the biological purpose of regulation of the *CDC9* gene by either DNA damage or during the cell cycle?

In addition to the *CDC9* gene, the *CDC8* (47, 286), *CDC21* (286), *POL1* (DNA polymerase 1) (111), and *RNR2* (ribonucleotide reductase) (47) genes are regulated during the cell cycle and show kinetics of induction essentially identical to that of *CDC9*. The DNA polymerase I transcript is also regulated during meiosis, showing about a 20-fold overall fluctuation (111). Also, there is evidence that, like the *CDC9*, *RAD2*, and *RNR2* genes, the *POL1* gene is DNA damage regulated. Following irradiation with 50 J/m^2, there was a threefold increase in the steady-state level of DNA polymerase I mRNA relative to irradiated cells, beginning about 30 min after irradiation and reaching a peak ~2 h later (111). These experiments were carried out with mid-log-phase cells, because the level of polymerase I mRNA in stationary-phase cells is too low for accurate measurement (111).

A detailed comparison of the sequences of the *RAD2* and *CDC9* upstream regions does not show significant similarities. In particular, the putative UAS identified in *RAD2* is not present in the *CDC9* noncoding region. Interestingly, however, the 5′ upstream sequence of the *CDC9* gene contains an extensive region of dyad symmetry (which unlike *RAD2* is not composed of AT-rich regions), and it has been suggested (12) that this potential stem-loop region may be involved in the regulation of expression of this gene. The hexanucleotide sequence ATGATT is repeated six times in the 650 bp upstream of the *CDC9* transcriptional start site (12). Clearly, it would be most interesting to evaluate the significance of this hexanucleotide and of the region of dyad symmetry by deletion analysis.

S. CEREVISIAE AS A MODEL FOR NUCLEOTIDE EXCISION REPAIR IN OTHER ORGANISMS

Rabbit antisera to Rad1, Rad2, Rad3, and Rad10 proteins have been raised against Rad proteins expressed in *E. coli* (186; H. Burtscher, A. J. Cooper, L. Naumovski, W. A. Weiss, and E. C. Friedberg, unpublished observations). In all cases the antisera react specifically with the appropriate Rad protein in extracts of yeast cells transformed with expression plasmids. The antisera have also been used to detect Rad2 and Rad3 protein expressed from single-copy chromosomal genes in untransformed cells and confirmed the evidence from transcriptional analysis and from expression of *RAD-lacZ* fusion genes that these *RAD* genes are very weakly expressed under constitutive conditions. Indeed, reactivity with Rad2 protein expressed from the single chromosomal gene could only be detected by immunoprecipitation (186).

These antisera, as well as the corresponding cloned genes, provide potentially useful experimental tools for exploring possible structural and functional relationships between nucleotide excision repair in yeasts and in other organisms. As indicated above, the yeast Rad10 and human Ercc1 proteins show considerable structural conservation (266). In an effort to explore a functional relationship between these proteins, the cloned *RAD10* gene was introduced into the UV-sensitive excision-defective CHO cell line UV20 and into several other cell lines from complementation group 2 of Thompson et al. (264), the genetic complementation group used to identify the human *ERCC1* gene (264). In initial experiments the *RAD10* gene was amplified (by cotransfection with the mouse *DHFR* gene) to optimize the amount of Rad10 protein expressed in these cells. *RAD10* transcripts were detected by Northern (RNA) blot analysis and Rad10 protein was demonstrated in both nuclear and cytoplasmic preparations of transformants with the amplified *RAD10* gene (C. Lambert, L. Couto, W. A. Weiss, R. Schultz, L. H. Thompson, and E. C. Friedberg, unpublished observations). Comparisons of the UV sensitivity of *RAD10*-bearing and control cells reproducibly showed partial complementation of the phenotypes of UV sensitivity or sensitivity to mitomycin C (Fig. 12). Similar results were obtained in UV-irradiated cells plated and quantitated after transient expression of the *RAD10* gene and in stable integrants containing nonamplified copies of *RAD10*. Thus, amplification of the yeast gene is not a prerequisite for this phenomenon, suggesting that the partial complementation of UV sensitivity reflects limitations inherent to interspecies complementation. The partial phenotypic complementation is specific. Integration and amplification of the *RAD3* gene in UV20 cells has no effect on UV sensitivity, and the *RAD10* gene has no effect on the UV sensitivity of CHO cells from other genetic complementation groups.

These results suggest that proteins for nucleotide excision repair may be conserved in many, if not all, eucaryotes and have motivated attempts to use yeast genes to isolate homologous genes from higher organisms. Direct probing of human genomic DNA with *RAD10* did not reveal specific hybridization by Southern blot analysis (cited in reference 58). However, both the *RAD10* and *ERCC1* genes hybridized to common fragments in the *Drosophila* genome, suggesting that the use of *Drosophila* probes may facilitate the isolation

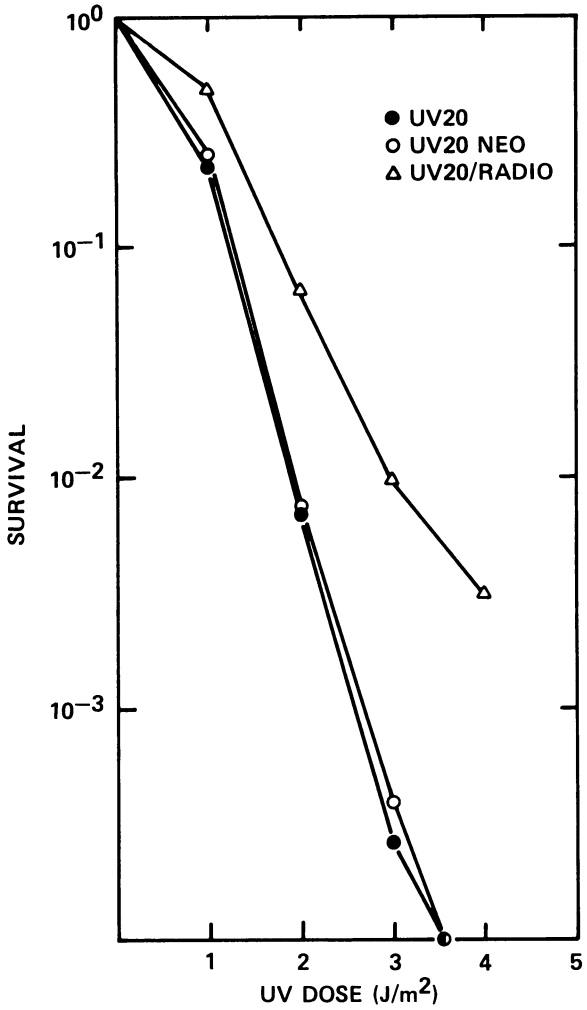

FIG. 12. Partial complementation of the UV sensitivity of the excision-defective UV-sensitive mammalian cell mutant UV20 by the yeast *RAD10* gene. UV20 NEO is a cell line transfected with a control plasmid without *RAD10* and was selected for stable integration of the marker *NEO* gene.

of human DNA repair genes (cited in reference 58). Probing digests of *Drosophila* DNA with the cloned *RAD1* and *RAD3* genes also yielded specific hybridizing fragments (cited in reference 58). These will presumably be used to search for homologous sequences in human DNA.

My colleagues and I have adopted a similar approach, using affinity-purified polyclonal antibodies purified from antisera to Rad proteins (see above). Antibodies against Rad3 protein react with two polypeptides in extracts of human HeLa cells (Naumovski and Friedberg, unpublished observations). These proteins (M_r, ~150,000 and ~95,000, respectively) may be distinct species or may be related. Clearly, the next step is to screen human expression libraries in the hope of identifying sequences that prove to be genes related to *RAD3*.

There is no evidence for structural or functional homology between yeasts and *E. coli* nucleotide excision repair genes or proteins. Antibodies against *E. coli* UvrA, UvrB, and UvrC proteins do not cross-react specifically with Rad proteins (C. Backendorf, W. A. Weiss, and E. C. Friedberg, unpublished observations). Similarly, antisera raised against the yeast Rad proteins fail to cross-react with extracts of untransformed *E. coli* cells or cells transformed with plasmids containing the cloned *uvr* genes. Also, neither transformation of *E. coli uvr* cells with plasmids containing cloned *RAD* genes nor the reciprocal experiment has resulted in phenotypic complementation (56).

EXCISION REPAIR OF CHEMICAL DAMAGE

This review is principally focused on the repair of DNA damage caused by UV radiation, since this is the most extensively studied model in yeasts. As indicated earlier, the organization of genetic loci for DNA repair into three largely nonoverlapping epistasis groups is based on sensitivity to killing by UV or ionizing radiation. However, there are indications that different epistatic interactions might emerge from a systematic survey of responses to chemical damage. Such systematic studies have not yet been reported, and the genetics of the excision repair of chemical damage to DNA is therefore not well established. These considerations notwithstanding, the following section presents a brief overview of cellular responses to chemical damage to DNA, with a particular emphasis on the psoralens and the cross-linking agent nitrogen mustard. Of necessity, the thrust of the succeeding paragraphs is on cellular-biological studies, reflecting the current paucity of molecular and biological information on this topic.

Repair of Psoralen Damage

Furocoumarins such as 8-methoxypsoralen (8-MOP) intercalate into DNA and, upon subsequent activation by UVA light (365 nm), form covalent pyrimidine adducts. The planar organization of the aromatic rings of these compounds facilitates reaction at one or both ends of the molecules, giving rise to monofunctional and difunctional adducts, respectively (192, 245, 255). In the latter situation a pyrimidine moiety in one of the DNA strands above the plane of the psoralen is covalently joined to a pyrimidine on the opposite strand situated below the plane of the compound, resulting in an interstrand cross-link. Only a fraction of the psoralen monoadducts can be photoactivated to form cross-links, and the ratio of monoadducts/cross-links in the DNA of cells treated in vivo depends on several parameters (192, 245, 255). Some furocoumarins have structures which do not permit the formation of interstrand cross-links in DNA. As seen later, these are particularly useful for the analysis of monofunctional damage.

Epistasis groups involved in repair of psoralen damage. Averbeck and Moustacchi (7) showed that yeast cells are sensitive to photoactivated 8-MOP, an agent which produces both monoadduct and cross-link damage in DNA. The *RAD3* epistasis group mutants (*rad1*, *rad2*, or *rad3*) were more sensitive than a *RAD6* group mutant (*rad9*), and a *rad2 rad9* double mutant showed additive sensitivity, suggesting that genes in both epistasis groups are involved in the repair of psoralen damage. The observation of additive rather than synergistic sensitivity has been interpreted as evidence for the action of two independent pathways on different substrates (82).

Comparisons between monofunctional and bifunctional psoralens indicate differences in sensitivity to killing in yeasts (174). When differences in reactivity with yeast DNA are accounted for, the observation that cells are more sensitive to 8-MOP than to the monofunctional analogs

3-carbethoxypsoralen and angelicin suggests that monofunctional base damage caused by psoralens is repaired (or bypassed) more efficiently than are interstrand cross-links. In contrast to the additive effect of killing by 8-MOP in a *rad2 rad9* double mutant, this mutant shows a synergistic response to killing by monofunctional psoralens, suggesting that for this type of DNA damage different repair pathways represented by the *RAD3* and *RAD6* epistasis groups act on the same lesion or repair intermediate (8).

The *RAD50* (recombinational repair) pathway mutants (see later) are also abnormally sensitive to killing by psoralens. However, the responses observed are in many cases dependent on whether cells are in exponential- or stationary-phase growth. In the exponential phase, *rad50*, *rad51*, *rad52*, *rad54*, and *rad55* mutants are very sensitive to both 8-MOP and 3-carbethoxypsoralen, while *rad57* and particularly *rad56* mutants are less so (85). In the stationary phase, the sensitivity of most of these mutants is decreased and, in particular, the difference between *rad56* and *rad57* mutants and wild-type strains treated with either compound is marginal. Curiously, the *rad55* mutant remains distinctly sensitive to the cross-linking agent 8-MOP. However, this mutant is insensitive to killing by the monofunctional agent 3-carbethoxypsoralen in stationary phase. A *rad53* mutant is insensitive to both psoralens in either growth phase (84).

These results are difficult to interpret. A priori, one might expect that the *RAD50* group mutants would not be abnormally sensitive to killing by monofunctional psoralens, since recombinational functions are not expected to be required for the repair of DNA damage produced by these agents. Hence, the possibility must be considered that the results reported by Henriques and Moustacchi (85) may reflect the presence of overlapping single-strand breaks in DNA generated during excision repair of closely spaced lesions on opposite strands. Indeed, in an independent study, Jachymczyk et al. (104) reported that a *rad51* mutant was insensitive to killing by angelicin under conditions of significant lethality by the cross-linking agent 4,5′,8-trimethylpsoralen.

In addition to genes in each of the three established epistasis groups, two new loci have been identified that are specifically required for the repair of psoralen damage in DNA, but not for the repair of pyrimidine dimers or damage caused by ionizing radiation. This was established by the isolation of the *pso2-1* and *pso3-1* mutant strains (84, 174). (The *pso1-1* mutant is not uniquely sensitive to psoralens and the mutant gene is probably allelic to a gene previously designated *rev3-1*, required for mutagenesis in yeasts [C. Cassier and E. Moustacchi, personal communication].) Both *pso2-1* and *pso3-1* mutants show enhanced sensitivity to killing by difunctional psoralens, but manifest a normal (or near normal) response to short-wave UV radiation and to gamma rays.

Mechanisms of repair of monofunctional and difunctional psoralen adducts. Attempts have been made to distinguish the mechanism of repair of psoralen monoadducts from that of cross-links in DNA. Jachymczyk et al. (104) observed a reduction in molecular weight of DNA, in both neutral and alkaline gradients, in cells incubated after treatment with a cross-linking psoralen. Based on these results, the authors concluded that the repair of cross-links involves double-strand breakage of DNA. No double-strand breaks were observed in the DNA of cells treated with monofunctional psoralen.

Double-strand breaks were detected at a significantly reduced level and rate in *rad3* mutants (104, 153), but were produced at normal levels in a *rad51* mutant. However, as expected from the known requirement for the *RAD51* gene in recombinational repair (65), such breaks were not repaired in this strain. Miller et al. (166, 167) showed that other genes in the *RAD3* epistasis group are involved in the repair of cross-links induced by 4,5′,8-trimethylpsoralen. Mutants carrying deletions of the *rad7* or *rad23* genes or both are deficient but not defective in the repair of cross-links (167), thus mimicking the phenotype observed during the repair of pyrimidine dimers (see above). The incision of DNA containing cross-links induced by treatment of cells with 4,5′,8-trimethylpsoralen also requires at least the *RAD1*, *RAD2*, *RAD3*, and *RAD4* genes (166). Thus, the evidence in *S. cerevisiae* suggests that repair of psoralen cross-links involves both excision and recombinational repair functions.

Mutants defective in the *PSO2* gene are able to incise DNA containing psoralen cross-links. However, like the *RAD52* epistasis group mutants, *pso2* mutants are apparently defective in the repair of double-strand breaks (153). However, unlike the genes in the *RAD52* epistasis group, the *PSO2* gene is not required for generalized recombination and apparently subsumes a special role in the repair of double-strand breaks produced exclusively in response to cross-link damage to DNA in yeasts (174). Consistent with this interpretation, *pso2* mutants are not abnormally sensitive to ionizing radiation (a DNA-damaging agent to which mutants defective in double-strand break repair are typically hypersensitive [see later]). Also, *pso2* and *rad52* mutants show other phenotypic differences and are not epistatic with respect to sensitivity to killing by the cross-linking psoralen 8-MOP (40).

Jachymczyk et al. (104) suggested that the repair of psoralen monoadducts occurs by the same excisional pathway as that used for photoproducts produced by 254-nm UV radiation. Consistent with this model, only single-strand breaks were observed during the incubation of cells treated with monofunctional psoralens, and *rad3* mutants were defective or deficient in the production of these breaks. However, Miller et al. (168) observed that the *rad3-2* mutant was not defective in DNA incision at sites of monoadduct psoralen damage and proposed that the sensitivity of *rad3-2* cells to monofunctional psoralens may reflect a requirement for Rad3 protein in postincision events.

A role for the *RAD3* gene and other nucleotide excision repair genes in the repair of psoralen monoadduct damage has been confirmed by using other experimental approaches. When cells are treated with 8-MOP plus UVA, a fraction of the monoadducts (those that are potentially cross-linkable) can be converted to cross-links by further irradiation (174). This alters the relative proportion of monoadducts and cross-links in the genome without changing the total yield of DNA damage caused by the psoralen. By administering a second dose of UVA light at different times after the first, it is possible to follow the fate of cross-linkable monoadducts (174). In wild-type cells such lesions are removed rapidly during incubation between the two doses of UVA radiation. Consistent with the role of nucleotide excision repair genes, the cross-linkable monoadducts persist in *rad2* and *rad3* mutants (174). During longer incubations following the first dose of UVA, excision-deficient cells are capable of carrying out at least one round of DNA replication and a fraction of the monoadducts in such cells is cross-linkable by a second dose of UVA. Thus, nonexcised monoadducts apparently do not constitute permanent blocks to DNA synthesis (174).

Like the repair of cross-link damage to DNA, cellular responses to psoralen monoadduct damage are not confined

to the nucleotide excision repair pathway. As indicated previously, mutants in the *RAD6* epistasis group (involved in both error-prone and error-free modes of DNA repair) are also sensitive to this form of DNA damage. Furthermore, *rad52* mutants are moderately sensitive to killing by 3-carbethoxypsoralen (83), suggesting that multiple pathways exist for the processing of psoralen monoadduct damage. Direct measurement of the loss of radiolabeled psoralen from the DNA of wild-type and mutant cells has shown that a triple mutant defective in the *rad2*, *rad6*, and *rad52* genes is still able to excise some psoralen monoadduct damage (83). This may reflect leakiness in one or more of the mutant alleles used or, more provocatively, it may reflect the existence of yet other (presumably minor) repair pathways for the repair of bulky base damage in *S. cerevisiae*.

Attempts to gain insights into the mechanisms by which psoralen damage to DNA is repaired have also utilized the traditional approach of searching for epistatic relationships between some of the *PSO* genes and the *RAD* genes from the three established epistasis groups (32, 40, 83, 86, 174). In general, the results of these experiments are complex and defy simplistic conclusions about the genetics of repair of psoralen damage to DNA in *S. cerevisiae*. Nonetheless, the following key points are to be noted.

(i) *PSO1*. The *pso1* mutation is epistatic to *rad6* for sensitivity to the cross-linking agent 8-MOP and to monofunctional psoralens. This relationship also holds for UV radiation at 254 nm and for exposure to ionizing radiation. Hence, the *PSO1* gene clearly can be classified in the *RAD6* epistasis group. However, as is true of the *RAD24* and *CDC8* genes, *PSO1* also fits into another epistasis group (Table 1). Thus, for example, *pso1* was found to be epistatic to *rad52* in cells treated with 8-MOP or ionizing radiation, but not with respect to UV radiation or monofunctional psoralen damage.

(ii) *PSO2*. Unlike *pso1* mutants, *pso2* mutants do not show epistatic interactions with *rad6* or *rad52* mutants for any agents tested, but do show epistasis with *rad3* for sensitivity to both monofunctional and difunctional psoralens. Thus, the *PSO2* gene is appropriately classified in the *RAD3* epistasis group and this supports a role for nucleotide excision repair functions in cellular responses to both forms of psoralen damage. Curiously, despite that *PSO1* belongs to the *RAD6* and *RAD52* epistasis groups and *PSO2* belongs to the *RAD3* epistasis group (Table 1), the *pso1* and *pso2* mutations are epistatic with respect to sensitivity to 8-MOP. Thus, the repair of psoralen cross-link damage apparently also involves the *PSO1* gene. However, they do not show epistasis for killing by monofunctional psoralens. Epistatic interactions of *pso3* mutants with the *pso1*, *pso2*, and *rad* mutants has not yet been reported.

To add a final note of complexity, there are indications that the mechanism(s) of repair of different forms of psoralen monoadduct damage may differ. When cells were treated with the monofunctional psoralen 7-methylpyrido-3,4-psoralen, single-strand breaks appeared in the DNA as in the case with other monofunctional psoralens. However, these breaks were very poorly repaired in Rad^+ cells, and this compound is much more cytotoxic than the other psoralens discussed (174).

Repair of Nitrogen Mustard Damage

Many of the UV-sensitive *rad* mutants isolated in the late 1960s and early 1970s were shown to be sensitive to killing by various chemicals, including the bifunctional alkylating agents nitrogen and sulfur mustard (26). In an effort to learn more about the repair of damage produced by these chemicals, Brendel and his colleagues searched for yeast mutants uniquely sensitive to them. Among several yeast mutants with enhanced sensitivity to nitrogen mustard, one mutation designated *snm1* (sensitive to nitrogen mustard) confers selective sensitivity to bifunctional alkylation of DNA (234, 235). This sensitivity extends to other cross-linking chemicals that are not alkylating agents, e.g., cisplatin and photoactivated psoralen (28). Subsequently (28), it was shown that the *snm1* mutation is in fact an allele of *PSO2* (Table 1). Thus, the requirement for the *PSO2* gene is apparently general for cross-link repair of DNA in yeasts. However, as pointed out by Siede and Brendel (249), one should be cautious about concluding that all cross-link damage in yeasts is repaired identically. The *RAD50* recombinational repair pathway mutants are clearly sensitive to killing by bifunctional psoralens (85); however, such mutants display a modest sensitivity to killing by nitrogen mustard in stationary-phase cultures (249). Thus, double-strand breaks may not constitute an important intermediate in the repair of cross-links induced by this particular chemical. Perhaps this refinement reflects the distinctive nature of the diguanyl cross-links produced by nitrogen mustard and the dithymidyl cross-links produced by photoactivated psoralen.

Parenthetically, it is interesting that the mutant phenotype of unique sensitivity to agents that cross-link DNA is not observed in *E. coli*, but is shared with human cells from the hereditary human disease Fanconi's anemia (see reference 55). This provides a further suggestive indication of the general conservation of repair responses in eucaryotes and their apparent distinction from repair mechanisms in lower organisms.

Repair of Simple Alkylation Damage

Extensive studies have been carried out on the sensitivity of yeast mutants to monofunctional alkylating agents (26, 37, 187, 199, 205). However, relatively little is known about the molecular biology or biochemistry of the repair of simple alkylation damage in *S. cerevisiae*. This is in stark contrast to the situation in *E. coli* and in mammalian cells, both of which have been shown to contain various DNA glycosylases which remove *N*-alkylpurines (such as 3-methyladenine, 3-methylguanine, and 7-methylguanine) from DNA (see reference 55). These enzymes generate apurinic or apyrimidinic sites which can be repaired by an excision mode involving the action of a specific class of enzymes called apurinic/apyrimidinic endonucleases. Similarly, the repair of O^6-alkylguanine in *E. coli* and in mammalian cells is mediated by the action of an enzyme called O^6-alkylguanine-DNA alkyltransferase. This enzyme catalyzes the removal of small alkyl groups from the O^6 position of guanine or O^4 position of thymine (and in *E. coli* also from phosphotriesters) and transfers alkyl groups to specific cysteine residues in the protein (see reference 55).

In *E. coli* the expression of the gene which encodes this alkyltransferase, as well as the gene for a well-characterized alkylation-specific DNA glycosylase, is regulated as part of a polycistronic complex that participates in the so-called adaptive response to alkylation damage (see reference 55). This response facilitates enhanced survival and decreased mutagenesis in bacteria exposed to monofunctional alkylating agents. In mammalian cells there is limited evidence for an analogous biological phenomenon (see reference 55). How-

ever, there is no convincing evidence for induced expression of the mammalian alkyltransferase enzyme following "adaptive" treatment of cells with alkylating agents.

The results of genetic and biochemical experiments suggest that *S. cerevisiae* does not mount an adaptive response to alkylation damage. Growth of cells in nontoxic concentrations of alkylating agents does not increase resistance to killing or mutagenesis following a challenge with higher doses. Also, there is no evidence for induction of an enzyme which resembles the alkyltransferase activity in *E. coli* or mammalian cells (152). Negative results were also obtained with constitutive yeast cells not exposed to "adapting" treatments with alkylating agents (152). These observations suggest that the repair of O^6-alkylguanine and O^4-alkylthymine in *S. cerevisiae* is mediated by a different mechanism(s). To test this suggestion directly, Maga and McEntee (152) measured the ability of cell yeast extracts to remove alkylated purines from DNA. They observed the removal of >90% of the O^6-methylguanine present in the DNA of cells treated with [^{3}H]methylnitrosourea when the DNA was incubated with extracts of *E. coli* containing active alkyltransferase. However, no removal was detected during incubation of DNA with extracts of yeast cells, regardless of the treatments to which the cells were exposed prior to disruption. Negative results are always open to multiple interpretations and it would be misleading to conclude definitively that alkyl adducts at the O^6 position of guanine are not repaired in yeast cells. However, the evidence to date does not provide any support for the presence of an alkyltransferase activity in yeast cells.

It should be noted that the adaptive response to alkylation damage is not totally ubiquitous, even in bacteria. This phenomenon has not been detected in *Salmonella typhimurium* (5) or in *Haemophilus influenzae* (119). It is not known how simple alkylation damage is repaired in these procaryotes. However, it has been well established that, in *E. coli*, larger alkyl groups in DNA (such as ethyl and butyl moieties) can be excised by the nucleotide excision repair pathway (267). As indicated earlier in this review, many *RAD3* epistasis group mutants are abnormally sensitive to killing by monofunctional alkylating agents, although the correlation between sensitivity to UV radiation and to specific monofunctional alkylating agents is limited. These observations, together with the failure to detect alternative pathways for alkylation damage repair, suggest that to the extent that such damage is repaired in yeast cells, it is processed by products of genes from the three established epistasis groups.

Further evidence in support of the role of nucleotide excision repair genes in the repair of alkylation damage comes from recent studies by Hoekstra and Malone (93). These investigators have shown that the *E. coli dam* gene (which encodes an enzyme that methylates the N^6 of adenine) can be expressed in yeast cells and results in the presence of detectable N^6-methyladenine in yeast chromosomal DNA. This methylation is associated with a moderate increase in mitotic recombination and mutation at some loci. Hoekstra and Malone (93) showed that wild-type, *rad6-1*, and *rad52-1* cells had identical levels of methylation detected by susceptibility of yeast DNA to the restriction enzyme *Dpn*I. However, DNA from cells defective in the *RAD1* or *RAD3* genes showed increased *Dpn*I sensitivity, suggesting reduced removal of methyl groups in mutants defective in nucleotide excision repair.

To learn more about the repair of simple alkylation damage in yeasts, it would clearly be useful to isolate mutants that are uniquely sensitive to such damage. A promising start in this direction stems from the recent isolation of a mutant designated *ngs1* (187) which is abnormally sensitive to killing by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, MMS, or ethyl methanesulfonate, but not to UV radiation. Recently, Kassir et al. (113) reported that a temperature-sensitive mutant in the *CDC40* gene (a gene required for normal passage through the cell cycle [114]) is very sensitive to treatment with MMS at the restrictive temperature. Incubation of cells at 37°C prior to or after MMS treatment at 23°C did not result in reduced survival relative to untreated cells. The mutant cells were also not particularly sensitive to UV radiation; however, it has not been firmly established that the *cdc40* mutant is uniquely sensitive to monofunctional alkylating agents. The phenotype of MMS sensitivity facilitated cloning of the *CDC40* gene by phenotypic complementation. The cloned gene has been mapped to chromosome IV between *trp4* and *ade8* (113). Analysis of the sensitivity of appropriate single and double mutants to MMS or UV radiation placed the *CDC40* gene in the *RAD6* epistasis group (130).

PHOTOREACTIVATION IN *S. CEREVISIAE*

S. cerevisiae was one of the earliest model systems for studying the genetics and biochemistry of enzymatic photoreactivation of DNA. DNA photolyase (originally called photoreactivating enzyme) was first described in extracts of yeast cells in 1960 (236; see reference 55). Early studies in cell-free systems demonstrated that the activity has a requirement for light at wavelengths of about 350 nm, and this observation, coupled with the results of action spectrum analysis, suggested that the yeast enzyme contains a chromophore that absorbs light at photoreactivating wavelengths. Since then there have been multiple reports on the purification of proteins considered to have DNA photolyase activity (see reference 55 for a review).

At present, there are indications of two apparently distinct DNA photolyases in yeast cells. One of these is a protein of M_r ~130,000, composed of two nonidentical subunits ~60 and ~85 kDa in size. Neither subunit alone is catalytically active; however, mixing the two restores DNA photolyase activity (21). Purification of this enzyme by affinity chromatography is consistently accompanied by a loss of activity, suggesting that a noncovalently bound chromophore may be removed during purification. A second enzyme of M_r ~53,000 has also been purified from yeast cells (103). Denaturation of this protein yields free flavine adenine dinucleotide, the presumed chromophore and the compound known to be present in DNA lyases purified from a number of procaryotes, including *E. coli* (45, 46, 237; cited in references 58).

PHR Genes

A mutant defective in a gene called *PHR1* was isolated by Resnick in 1969 (213). He mutagenized a UV-sensitive *rad2* strain defective in excision repair of UV-irradiated DNA and screened for mutants which failed to show improved growth following exposure to UV irradiation and subsequently to photoreactivating light. Using a DNA transformation assay, extracts of the mutant (designated *phr1*) were shown to be defective in DNA photolyase activity (216). Subsequently, a second *phr* mutant was independently isolated (148). This strain is also apparently defective in DNA photolyase activity, but the mutation is believed to be an allele of *phr1*, since it fails to complement the UV sensitivity of the latter. A gene designated *PHR2* was identified in yet another mutant defi-

cient (but not defective) in photoreactivation in vivo and in DNA photolyase in vitro. Genetic analysis suggests that the *PHR1* and *PHR2* genes are distinct, but are closely linked, with a separation of only 18 centimorgans (148).

The *PHR1* gene has been cloned in several laboratories (242, 293) by phenotypic complementation of the *phr1* mutation. Both the cloned gene and the chromosomal allele have been independently mapped to the right arm of chromosome XV, distal to the *PRT1* gene. When the cloned gene was tailored into an *E. coli* expression vector containing the *tac* promoter and transformed into a mutant of *E. coli* defective in DNA photolyase, complementation of the Phr^- phenotype was observed (239). Hence, it is likely that the *PHR1* gene is a structural gene that encodes a yeast DNA photolyase. These complementation studies also suggest that the enzyme encoded by the *PHR1* gene normally contains a neutral flavin free radical similar to that present in the *E. coli* enzyme (237), since *E. coli* is the only possible source of a chromophore for the protein expressed from the cloned yeast gene. In a reciprocal experiment, it has also been shown that the cloned *E. coli phr1* gene complements the phenotype of the yeast *phr1* mutant (131).

The yeast *PHR1* gene contains an ORF of 1,695 bp (240, 293) and could encode polypeptides of calculated molecular weights ranging between 57,200 and 66,200, depending on which of several potential translational start sites are used in vivo (240). These size ranges together with the complementation results just described suggest that the yeast *PHR1* gene encodes the DNA photolyase of M_r ~53,000 mentioned above.

The amino acid sequences of the cloned yeast and *E. coli* genes show considerable similarity (240, 293). Overall, there is 36.2% identity; however, two short regions near the amino and carboxy termini of the two polypeptides show even greater similarity. These features suggest that the yeast and *E. coli* enzymes possess common structural and functional domains involved in the binding of substrate or chromophore or both (240). Despite the conservation of amino acid sequence, the cloned *E. coli* and yeast genes do not cross-hybridize and neither gene has provided a useful probe for the detection of homologous sequences in human or chicken genomic DNA (164).

At the present time, there is no satisfactory genetic basis for a second DNA photolyase from *S. cerevisiae*, the putative heterodimeric protein of M_r ~130,000. If this represents a biologically important enzyme, it is not clear why *phr1* mutants are apparently totally defective in DNA photolyase activity. The significance of the *PHR2* gene is also not understood. In view of the fact that it is genetically linked to *PHR1*, one must consider the possibility that this gene has a regulatory role that governs the expression or activity or both of the enzyme encoded by the *PHR1* gene. The molecular cloning of *PHR2* as well as detailed studies on the regulation of *PHR1* are expected to shed some light on this complex situation.

RAD52 EPISTASIS GROUP

Yeast mutants have been isolated that are abnormally sensitive to killing by ionizing radiation. As indicated earlier, mutants primarily sensitive to this agent are designated with locus numbers from *RAD50* upwards. The genetics and phenotypic characterization of these mutants have been described in detail in other publications (65, 82, 241) and it is not my intention to retread this ground in any depth. A consideration of the *RAD52* and the *RAD6* (see later) epistasis groups is included here largely for the sake of completeness and in the interests of providing a basis for comparison with the more detailed information currently available on excision repair of DNA in *S. cerevisiae*. Also, in recent years several genes in the *RAD52* and *RAD6* epistasis groups have been isolated by molecular cloning, and information gleaned from these molecular studies has not been comprehensively reviewed elsewhere.

Among the *RAD52* group, the *rad51*, *rad52*, and *rad54* mutants are extremely sensitive to ionizing radiation. These three *rad* mutants are almost completely defective in meiotic and mitotic recombination (65, 82, 241). In addition, these mutants have the phenotype of both spontaneous and radiation-induced chromosome instability, leading to monosomy in diploids (169, 170), and are defective in the repair of double-strand breaks (91, 215) and homothallic switching (154). Finally, these mutants show a sharp reduction of the X-ray resistance normally associated with mating-type heterozygosity (see reference 65).

The *rad50*, *rad53*, *rad55*, *rad56*, and *rad57* mutants are also classified in the *RAD52* epistasis group, although they are typically less sensitive to ionizing radiation than the *rad51*, *rad52*, and *rad54* mutants discussed above (65). These mutants show other phenotypic differences that distinguish them from the *rad51*, *rad52*, *rad54* group (65). Thus, for example, homozygous diploids are typically more resistant to X rays than the corresponding haploids. Moreover, these mutants are not defective in the enhanced X-ray resistance associated with mating-type heterozygosity and show rates of X-ray-induced mitotic recombination higher than those of *rad51*, *rad52*, and *rad54* mutants (see reference 65). It has also been reported that the *rad50-1* mutant is proficient in the repair of double-strand breaks (see reference 65). However, the status of double-strand break repair in other members of this subgroup has not been documented. The observation of epistatic interactions among all of these mutants, coupled with phenotypic differences between the *rad51*, *rad52*, and *rad54* mutants on the one hand and the *rad50*, *rad53*, *rad55*, *rad56*, and *rad57* mutants on the other, has led to the hypothesis that the former group of loci mediate steps common to all repair functions mediated by the latter, but that the latter mediate different subclasses of repair events dependent on the former (65).

The observation that the *RAD52* group mutants are defective in meiosis suggests that the repair of ionizing radiation damage shares biochemical events in common with elements of DNA metabolism in meiosis. There is extensive experimental evidence that normal meiosis has a requirement for recombinational events. This is certainly consistent with the phenotypes of the *rad52* mutants summarized above and has led to the implicit assumption that recombination is a key metabolic transaction during the repair of ionizing radiation damage. Indeed, the *RAD52* epistasis group of genes is thought to reflect the existence of a recombinational repair pathway in yeast cells fundamentally distinct from the nucleotide excision repair pathway operating through the *RAD3* group of genes. (Recombination events may also be required for postreplicative repair of UV radiation damage, thus providing a reasonable explanation for the observation that some *RAD52* epistasis group mutants are also slightly sensitive to UV radiation [118].)

There is limited information on the biochemistry of so-called recombinational repair in *S. cerevisiae*. Operating on the premise that genes involved in genetic recombination might encode or regulate deoxyribonucleases, Chow and Resnick (34) measured general nuclease activity in extracts

of *RAD* and various *rad* mutant cells and found no significant differences in the specific activity of both Mg^{2+}-dependent and Mg^{2+}-independent nucleases active on double- or single-stranded DNA. The yeast extracts were also examined for cross-reaction with an antiserum raised against a nuclease activity from *N. crassa*. Immunoprecipitation reactions showed a reduction in total nuclease activity in extracts of wild-type cells. However, when extracts of *rad52* mutant cells were examined for the presence of antiserum-precipitable nuclease, none was detected, despite the fact that the level of total nuclease activity in *rad52* cells was not reduced (34). Transformation of *rad52* cells with a recombinant plasmid containing the cloned *RAD52* gene (see later) restored the nuclease activity. The antibody-precipitable nuclease activity contributed as much as 50% to the increase in total alkaline deoxyribonuclease activity during meiosis in yeast cells, beginning prior to the time of meiotic DNA synthesis and extending into the period of commitment to recombination (214, 218).

The antibody-precipitable nuclease activity has an apparent M_r of ~70,000 based on its electrophoretic migration in denaturing polyacrylamide gels (214). However, the size of the *RAD52* gene product is expected to be ~56 kDa based on the size of the cloned gene, suggesting that *RAD52* is not the structural gene for this nuclease. More recently, Chow and his colleagues have isolated the gene that encodes this yeast nuclease by screening a yeast expression library with the *N. crassa* antibodies. Transformation of yeast cells with a multicopy plasmid containing a recombinant insert identified by this screening resulted in the presence of 3 to 10 times more cross-reacting material and 1.5 to 2 times more immunoprecipitable nuclease activity (Chow, personal communication). Such cells (in stationary phase) also manifested an increased resistance to MMS relative to untransformed cells. However, this increased resistance was not observed in transformed *rad52* mutant cells (Chow, personal communication).

As indicated earlier, these data represent a second example of the loss of enzymatic activity in extracts of cells defective in a gene distinct from the coding gene for the enzyme in question. The other example is the inability to detect ATPase III activity in extracts of *rad3* cells (see above).

Molecular Cloning of Genes in the *RAD52* Epistasis Group

***RAD50* gene.** The *RAD50* gene has been cloned on a multicopy plasmid by phenotypic complementation of the sensitivity of a *rad50-1* mutant to MMS (126). A plasmid containing a 3.9-kb insert restored the resistance of the *rad50-1* mutant to MMS as well as to ionizing radiation. Also, normal levels of UV-induced mitotic recombination were restored and transformants sporulated normally. Preliminary mapping of the cloned gene following chromosomal integration of a 2μ-based recombinant plasmid and loss of chromosomal markers suggests that it is present on the left arm of chromosome XIV (126). This was confirmed by conventional genetic analysis, which placed the revised location of the gene near *pet2* on chromosome XIV (126).

The cloned gene hybridizes to a 3.3-kb transcript present in very low amounts in vegetative cells (125). No significant increase in the amount of this transcript was detected in cells exposed to UV radiation or MMS. Recent sequencing of the *RAD50* gene revealed an ORF containing 1,312 codons that could encode a polypeptide of calculated M_r ~157,000 (N. Kleckner, personal communication). The amino terminus of the putative polypeptide shows significant similarity to purine nucleotide-binding domains observed in the amino acid sequences of myosin, G proteins, and adenylate kinase (Kleckner, personal communication).

Disruption of the chromosomal *RAD50* allele following integration of an internal fragment of the cloned gene by homologous recombination is not lethal to haploid cells (125). Hence, *RAD50* is not an essential gene. This result is consistent with earlier observations that many *rad50* mutant alleles contain suppressible nonsense mutations. Indeed, the cloning of *RAD50* by phenotypic complementation has been complicated by the isolation of a large number of plasmids which turned out to contain suppressor genes rather than *RAD50* (126, 241).

***RAD52* gene.** The *RAD52* gene was also isolated by phenotypic complementation of appropriate mutants (2, 243). Analysis of the nucleotide sequence of the cloned gene (2) revealed a single ORF of 1,512 nucleotides. Transcriptional mapping showed the presence of two transcripts with different 3′ ends. The precise transcriptional and translational start sites have not been determined. The identification of the latter is complicated by the presence of five in-frame ATG codons within a region of 118 nucleotides. Assuming for the moment that the first ATG codon in the *RAD52* ORF is indeed the codon for translational initiation, the gene is expected to encode a polypeptide of calculated M_r 65,100 (Table 2). The protein has a calculated net charge of +3 at neutral pH (Table 2). Several hydrophobic domains are noted in the amino-terminal third of the protein (Fig. 11). Gene disruption experiments have shown that *RAD52* is also not an essential yeast gene (243).

***RAD51*, *RAD54*, *RAD55*, and *RAD57* genes.** Recombinant plasmids containing the *RAD51*, *RAD54*, *RAD55*, and *RAD57* genes have been isolated by phenotypic complementation (241). The *rad54-3* mutant is temperature sensitive and shows enhanced killing by X rays at 36°C relative to that observed at 23°C. The cloned gene fully complements the phenotype of the *rad54-3* mutant at both temperatures. Like *RAD50* and *RAD52*, the *RAD54* gene is not essential. *RAD54* is inducible by treatment of cells with DNA-damaging agents, including X rays, UV light, and MMS, and all three agents show a dose-treatment effect on induction (35). In addition, the *RAD54* gene can be induced by double-strand breaks in DNA generated by regulated overexpression of the cloned gene encoding the restriction enzyme *Eco*RI (35). Expression is enhanced 3- to 12-fold as determined by mRNA levels and by β-galactosidase activity, using *RAD54-lacZ* fusions (35). Induction of *RAD54* appears to be independent of cell cycle effects (35). It is clearly of interest to determine whether regulation of the two damage-inducible repair genes *RAD54* and *RAD2* (which are also not cell cycle regulated) is mediated by common determinants.

There are indications that the *RAD52* gene may be very weakly inducible by DNA-damaging agents (35). The status of the *RAD50* gene is undetermined at the time of writing. The *rad55-3* and *rad57-1* mutants are cold sensitive, and this phenotype is fully complemented by a number of recombinant plasmids. Genetic analysis has established that plasmids which complement *rad55-3* do indeed contain the *RAD55* gene. A similar analysis has not yet been reported for plasmids containing the putative *RAD57* gene (241).

RAD6 EPISTASIS GROUP

Loci in the *RAD6* epistasis group affect the sensitivity of mutants to both ionizing and UV radiation (65, 82). Of the

three epistasis groups described for *S. cerevisiae*, the correlation between the *RAD6* group as a whole and a specific biochemical pathway by which cells respond to DNA damage is the least obvious and poses the most extensive challenge to the general utility of epistatic analysis. None of the mutations in this epistasis group affect mitotic recombination except *rad9* (122), and none affects meiosis except *rad6*, which prevents sporulation (39).

An interesting property of at least some of the mutants in this group is that of defective mutagenesis following exposure to UV radiation. By analogy with the *recA lexA* pathway of inducible error-prone repair of DNA in *E. coli*, it has been suggested that the *RAD6* group of genes is involved in error-prone repair in *S. cerevisiae* (136, 142, 206). However, it is generally acknowledged that such analogies are at best gross oversimplifications and at worst totally invalid. McKee and Lawrence (161) have suggested that loci in the *RAD6* epistasis group can be subdivided into four distinct categories based on phenotypic differences. *RAD6* exists in a category of its own. As indicated above, it is required for the error-prone repair of UV radiation damage and of damage produced by most mutagens thus far examined. However, *RAD6* is also believed to be involved in an error-free mode of repair of UV radiation damage. A second category includes *RAD18*, which appears to be concerned with *RAD6*-dependent error-free repair of DNA damage. The third category includes *RAD9* and *RAD15*, and a fourth category includes the *REV* loci, which appear to be largely concerned with error-prone repair. Even in this last-mentioned subgroup there is evidence for involvement in a nonmutagenic error-free component of DNA repair (250).

The molecular mechanisms of mutagenesis in yeast cells are poorly understood, and the search for an illusory RecA-like protein has been plagued with failures. The interested reader is referred to a comprehensive review (135) for further information on some aspects of this complex topic. To date, of the multiple genes in this epistasis group only the cloning of the *RAD6* gene has been reported in the literature (127, 204). Among other problems, this presumably reflects the fact that many of the mutants represented in this group lack suitable selectable phenotypes for screening of yeast genomic libraries.

RAD6 Gene

The *RAD6* gene was cloned by complementation of the UV sensitivity of the mutant *rad6-1* (127, 204). Plasmids containing the cloned gene restore wild-type levels of resistance to UV and gamma radiation, and to MMS, in both *rad6-1* and *rad6-3* mutants. Disruption of the *RAD6* gene in a diploid strain generates viable haploid spores. Hence, the *RAD6* gene is not essential. However, when *rad6* null mutants are grown exponentially, a significant proportion (~45%) of the cells are arrested at the stage of medial nuclear division (127), suggesting that *RAD6* may play an important role at a specific stage of the cell cycle. While this role is apparently not essential, its absence causes cells to grow more slowly and contributes to cell lethality.

The *RAD6* gene encodes two transcripts of 0.98 and 0.86 kb, which differ in their 3′ termini. The *RAD6* ORF consists of 516 nucleotides and could encode a protein of calculated M_r 19,704, assuming that translation initiates at the first ATG codon (222). However, there are three in-frame ATG codons in the first 50 nucleotides of the gene. Examination of a hydrophilicity plot of the putative Rad6 polypeptide reveals pronounced hydrophilic amino- and carboxy-terminal regions. However, the middle third of the polypeptide is quite hydrophobic (Fig. 11). The last 23 codons specify 20 acidic amino acids, including 13 consecutive Asp residues (Fig. 5) (222). In fact, half of the total acidic amino acid residues in the polypeptide are concentrated in this region. It has been pointed out (222) that this highly acidic carboxy terminus is reminiscent of the high-mobility group proteins HMG-1 and HMG-2 associated with histones, and it has been suggested that Rad6 protein may play an important role in nucleosome assembly and disassembly in *S. cerevisiae*.

Very recently, it has been directly shown that Rad6 protein has a ubiquitin-conjugating activity that catalyzes the ubiquination specifically of histones H2A and H2B (107). This fascinating observation stems from the isolation of a series of ubiquitin-conjugating proteins from yeast cells by affinity chromatography. Comparison of the amino acid sequence of a peptide fragment of one of these proteins with a protein data base revealed identity with a portion of the translated sequence of the *RAD6* gene. When the cloned gene was overexpressed in *E. coli*, extracts were shown to specifically catalyze the ubiquination of histones H2A and H2B. Jentsch et al. (107) speculate that ubiquination of histones may play an important role in chromatin remodeling during DNA repair, either by selective degradation or by conformational alteration of these basic proteins.

It has been reported that expression of *RAD6* is regulated in the cell cycle (129). During late S/early G2 phase of the cycle, increased levels of *RAD6* mRNA are observed. Increased levels of *RAD6* transcripts have also been detected in cells treated with MMS (129). But it is suggested (129) that this may result from temporary arrest of cells in G2 (128, 291) rather than a specific response to DNA damage.

MISMATCH REPAIR OF DNA

This review would be incomplete without a few comments on the topic of mismatch repair in *S. cerevisiae*, a rapidly emerging area in the field of DNA repair. Mismatched nucleotides in the genome can be generated as a result of replicative infidelity during semiconservative DNA synthesis, as the result of spontaneous base damage such as the deamination of cytosine to generate uracil in DNA, or during recombination (176). The latter situation leads to the formation of heteroduplex DNA molecules which result in non-Mendelian segregation of alleles during meiosis, detectable by conventional tetrad analysis in fungi. Classical models of genetic recombination (95, 97, 165) suggest that mismatch correction of a single heteroduplex molecule will cause a deviation from Mendelian 4:4 segregation of tetrads, resulting in gene conversion and hence in 6:2 and 2:6 segregations (when meiotic tetrad segregation ratios are presented as octads) (Fig. 13). If, on the other hand, the heteroduplex is not corrected during meiosis, postmeiotic DNA synthesis at the first mitotic division will result in 5:3 and 3:5 segregation of alleles (so-called postmeiotic segregation) (Fig. 13). While other explanations for these aberrant segregations are tenable (261), recent genetic, biochemical, and molecular evidence supports the concept of mismatch correction of heteroduplexes in *S. cerevisiae*.

The model of mismatch correction summarized above predicts that strains with high levels of postmeiotic segregation are deficient or defective in mismatch correction and vice versa. Indirect evidence consistent with this prediction was recently reported by White et al. (284), who demonstrated a striking correlation between mismatches predicted for high postmeiotic segregation frequency alleles in *S.*

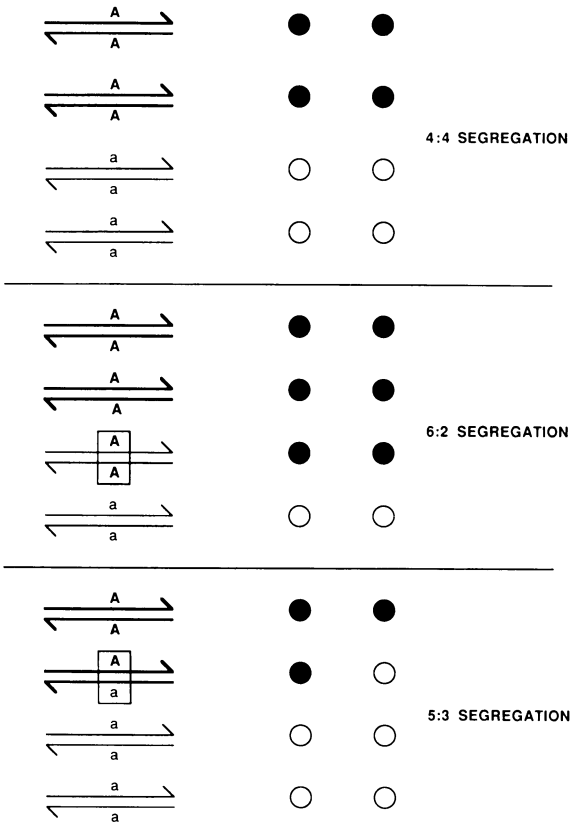

FIG. 13. Diagrammatic representation of meiotic events by analysis of tetrads. Spore segregation analyses are presented as octads for the sake of clarity. Normal meiosis results in 4:4 segregation of the alleles A and a (top). If a mismatch is generated in one of the DNA strands during meiotic recombination, the heteroduplex A/a results (bottom). Repair of this heteroduplex can yield the homoduplex A/A (boxed region in middle), resulting in 6:2 postmeiotic segregation of the alleles. However, if the heteroduplex is not repaired (bottom), the alleles will segregate 5:3.

cerevisiae and mismatches repaired with low efficiency in the procaryote *Streptococcus pneumoniae*. Fogel and his colleagues (289; cited in reference 176) have isolated a series of mutants which manifest a high frequency of postmeiotic segregation (designated *pms*). These mutants are presumed to identify a cellular function(s) required to maintain low postmeiotic segregation frequencies at many heterozygous sites in the genome. Consistent with a postulated requirement of wild-type *PMS* alleles in mismatch repair of DNA, mutants manifest a mutator phenotype (289). Also, in mutant diploids, spore viability is reduced, and among survivors gene conversion and *PMS* frequencies are increased, although the frequency of reciprocal exchanges is unaffected.

At the biochemical level, Kolodner and his associates have recently explored mismatch correction of exogenous heteroduplex DNA both in vivo and in a cell-free system. The former experimental system utilized transformation of yeast cells with plasmids containing 8- or 12-bp insertion mismatches or A · C or C · T single-base-pair mismatches (19). Analysis of the products of these transformations was consistent with mismatch repair of DNA. More recently, altered mismatch correction in the mutant strain *pms1-2* was demonstrated following transformation with heteroduplex plasmid DNA (20), thus providing further evidence in support of the role of the *PMS1* gene in mismatch correction in *S. cerevisiae*.

For cell-free studies on mismatch repair in *S. cerevisiae*, Muster-Nassal and Kolodner (176) constructed heteroduplex DNA substrates with 4- or 7-bp insertion/deletion mismatches or with each of the eight possible single-base-pair mismatches. Extracts of mitotic cells catalyzed the correction of mismatches in a reaction that requires Mg^{2+} and that demonstrates a partial requirement for adenosine 5′-triphosphate and the four usual deoxynucleoside triphosphates. A · C and G · T single-base-pair mismatches, as well as the insertion/deletion substrates were repaired efficiently. However, the other six single-base-pair mismatches were poorly repaired. Mismatch correction was accompanied by detectable repair synthesis of DNA, resulting in patches of <20 nucleotides located at or near the sites of the repaired mismatches.

The establishment of both in vivo and in vitro model systems for the repair of heteroduplex DNA and the potential for examining their biological validity by using the *pms* mutants already isolated promise an exciting explosion of molecular and biochemical information on mismatch repair in yeast cells in the near future. Among other anticipated results, the expected availability of cloned *PMS* genes and their overexpression to yield proteins will provide an alternative avenue for detailed biochemical studies and should provide important confirmation for the cell-free studies already in progress.

CONCLUSIONS

At the outset, apologies may be in order for the immodest length of this review. Nonetheless, this sober fact coupled with the recognition that many aspects of cellular responses to DNA damage in yeast cells have been deliberately deleted, or at best treated in a cursory manner, attest to the extensive literature that has accumulated on this topic, particularly in the last decade. The advent of the "new genetics" in *S. cerevisiae* and particularly the elegant application of recombinant DNA technology to this eucaryote have paved the way for impressive progress in our understanding of how *S. cerevisiae*, specifically, and perhaps eucaryotes generally, handle genomic insult.

Certainly in the area of nucleotide excision repair of base damage produced by UV radiation (the major theme of this review), the future is clear. It is simply a matter of time before the Rad1, Rad2, Rad3, Rad4, and Rad10 proteins are purified to physical homogeneity and are available for (hopeful) reconstitution of a partial, if not complete, incision/excision complex in vitro. It seems reasonable to expect that these five proteins will carry out at least some elements of nucleotide excision repair, using UV-irradiated (or otherwise damaged) DNA or chromatin or both as model substrates. Of course, it will be most interesting to determine what else might be needed in such a cell-free system. Thus, regardless of the results obtained from such experiments, pursuing the isolation of other genes from the *RAD3* epistasis group, as well as the products they encode, is mandatory. This will not be an easy task. The available *rad7*, *rad14*, *rad16*, *rad23*, *rad24*, and *mms19* mutants are not very sensitive to UV radiation or to any other form of exogenous DNA damage thus far examined. Hence, the well-tested and well-worn strategy of cloning by phenotype complementation is not likely to be generally useful with these mutants and alternative strategies will have to be developed.

The same general thrust with the *RAD52* and *RAD6* groups of genes is likely to be equally successful, although the lack of suitable complementable phenotypes appears to be impeding substantial progress with the latter. This is particularly lamentable, because there is a general paucity of information on the molecular mechanisms and biochemistry of mutagenesis in all biological systems. The progress with the *RAD52* group of genes is impressive and promises to yield considerable general information about meiotic and mitotic recombination in yeasts, in addition to specific information about postreplicative recombinational repair modes in damaged DNA.

It is highly likely that the molecular mechanism of the excision repair of bulky chemical adducts from yeast DNA is fundamentally similar to that of bulky photoproducts. However, my review of the literature has convinced me that there are probably subtle (and also not so subtle) differences. Clearly, much remains to be learned about the repair of various forms of cross-linking in DNA, and the emergence of the *PSO2* and *PSO3* genes has added surprising and interesting complexity to the general problem of nucleotide excision repair in *S. cerevisiae*.

It would appear that yeast cells do not mount an adaptive response to simple alkylation damage, nor do they use specialized repair reactions for O^6 and O^4 alkylations. It certainly would be useful to know whether *S. cerevisiae* is unique in this respect or whether this situation is common to other yeasts and fungi.

The demonstration that a number of yeast DNA repair genes are induced in cells exposed to DNA damage is interesting. I do not anticipate the emergence of a single regulatory network with an organizational simplicity similar to that of the SOS system in *E. coli*. The evidence to date suggests to me that DNA repair genes will prove no exception to the general diversity of regulatory mechanisms demonstrated for other genes in *S. cerevisiae*. In addition, it is highly probable that many genes induced by DNA damage are functionally unrelated to the processing of that damage, and students of induction by DNA damage in yeast cells may find themselves wandering into areas that bear no relationship to DNA repair.

Finally, I would stress that there can never be too much genetics in this game. If indeed, as I strongly suspect, the genetic framework for cellular responses to DNA damage is still incomplete, we must isolate more and more mutants. And to achieve this goal, we must resist the short-sighted attitude that such labors are not the stuff of 21st century scientists. Similarly, the new generation of students and postdoctoral fellows who have cut their scientific eyeteeth on Southern blots and gene cloning must learn that, while isolating genes is both elegant and facile, the ultimate answers to fundamental biochemical problems such as the mechanisms of replication, transcription, recombination, and repair of DNA lie not so much with these units of genetic information as with the protein products they encode.

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