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RADH, a gene of *Saccharomyces cerevisiae* encoding a putative DNA helicase involved in DNA repair. Characteristics of radH mutants and sequence of the gene

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ABSTRACT

A new type of radiation-sensitive mutant of *S. cerevisiae* is described. The recessive *radH* mutation sensitizes to the lethal effect of UV radiations haploids in the G1 but not in the G2 mitotic phase. Homozygous diploids are as sensitive as G1 haploids. The UV-induced mutagenesis is depressed, while the induction of gene conversion is increased. The mutation is believed to channel the repair of lesions engaged in the mutagenic pathway into a recombination process, successful if the events involve sister-chromatids but lethal if they involve homologous chromosomes. The sequence of the *RADH* gene reveals that it may code for a DNA helicase, with a Mr of 134 kDa. All the consensus domains of known DNA helicases are present. Besides these consensus regions, strong homologies with the Rep and UvrD helicases of *E. coli* were found. The RadH putative helicase appears to belong to the set of proteins involved in the error-prone repair mechanism, at least for UV-induced lesions, and could act in coordination with the Rev3 error-prone DNA polymerase.

INTRODUCTION

The different DNA repair mechanisms are mediated by proteins that act in a coordinated way and often as enzymatic complexes. For instance, the first steps in the error-free repair of ultraviolet-induced lesions involve in *Escherichia coli* the *UvrA*, *B*, *C* and *D* gene products (1) and in *Saccharomyces cerevisiae* the *RAD1*, *2*, *3*, *4* and *10* gene products (2). *UvrD* (3) and *RAD3* (4) encode DNA helicases. In the corresponding mutants, the excision of lesions is impaired but the mutational and recombinational mechanisms themselves seem unaffected. This suggests the existence of other helicases involved in these repair processes and indeed, for *E. coli*, one of the recombination pathways involves the RecBCD enzyme complex which was shown to possess, besides the exoV nuclease activity (5) an helicase activity (6).

Here we describe a yeast gene that encodes a putative DNA helicase involved in mutagenic repair, at least for UV-induced lesions. The gene was cloned by complementation of a new type of radiation-sensitive mutant, called *radH*, originally isolated as a suppressor of the high radiation sensitivity of *rad18*-deleted mutants, *RAD18* gene being involved in the error-prone repair. The isolation and characteristics of the *radH-rad18* double mutant cells will be described elsewhere. When the *radH* mutation was transferred into a RAD18$^+$ context, it was found to be recessive, and to confer a radiation-sensitive phenotype. We report now the properties of the *radH* mutant leading to the conclusion that the mutation affects the error-prone repair of UV-induced lesions, and in a second section, the sequence of the gene which codes for a putative DNA helicase.

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MATERIALS AND METHODS

Yeast strains are listed in Table 1. *E. coli* strains were MC1066, HB101, TG1 and for phage production JM101. For yeast cells, complete (YEP) or minimal medium (MM) supplemented with the desired elements (7) were used. For mutant selection, canavanine (20 μg/ml) was added to minimal medium. LB or M9 (8) were the media for bacteria.

The procedures used for genetic constructions and analysis, transformation, plasmid preparations, subclonings and gel electrophoresis were the standard ones (7,8,9).

For sequencing, DNA fragments obtained from the YRP7-S plasmid (Figure 2) were cloned into PTZ18R and PTZ19R plasmids. Overlapping deletions were obtained by DNase1 treatment (10). The dideoxy sequencing technique was used (11).

For UV (260 nm) and gamma-ray (^{60}Co) irradiations, the cells were suspended in 0.9% NaCl except for UV mutagenesis experiments for which irradiation was applied on the plated cells. Dosimetry was determined by a Latarjet UV dosimeter, and, for gamma-rays, by ferrous sulfate solutions. All the experiments were repeated several times.

RESULTS

UV and gamma-ray responses of the radH mutant.

UV response of the radH haploids. The *radH* haploids are UV sensitive, but only if irradiated in the G1 mitotic phase. In Figure 1, panel A, the survival curves of logarithmic and stationary phase cells are shown. Estimates of the proportion of G2 cells in each population, based on microscopical observation of the cell morphology (12) are 30% and 5% respectively and correspond roughly to the proportion of resistant cells deduced by extrapolation from the survival curves. That this G2 repair is due to recombinational repair is indicated by the effect of the *rad50* mutation coupled with the *radH* mutation : it abolishes the G2 repair (Figure 1, panel B). The *rad50* mutation prevents, after X-rays, recombinational repair, notably between sister-chromatids (13).

The induced mutagenesis is severely depressed by the *radH* mutation. This was found with different mutational systems. In Figure 1, panel C, is shown the induction of forward

Table 1. Yeast strains

Strain	Genotype
C6378	*Mata leu2 trp1 ura3 ade5 can1*
FF1866	*Mata leu2 trp1 ura3 ade5 can1 rad18::LEU2*
FF1889	*Mata leu2 trp1 ura3 ade5 can1 rad18::LEU2 radH*
FF18244	*Mata leu2 trp1 ura3 ade5 can1 radH*
FF1848	*Mata/Matα* 2X(*leu2 trp1 ura3 ade5 can1*)
FF18341	*Mata/Matα radH/radH* 2X(*leu2 trp1 ura3 ade5 can1*)
FF18404	*Mata/Matα RADH/radH* 2X(*leu2 trp1 ura3 ade5 can1*)
ZZ2	*Mata leu2 trp1 ura3 ade5 rad50-1*
ZZ14	*Mata leu2 trp1 ura3 ade5 his7 rad50-1 radH*
FF18266	*Matα leu2 ura3 his7-1 lys2-1*
FF18330	*Mata leu2 trp1 his7-1 radH*
FF18700	*Mata/Matα his7-1/his7-2 RADH/radH*
FF18701	*Mata/Matα his7-1/his7-2*

The strain C6378 was given by G. Simchen. The first seven strains constitute an isogenic series constructed by classical methods including mating-type switch induced by a HO-containing plasmid and genomic deletion of *RAD18*. Only the relevant genotype is indicated for the last two strains. The *leu2*, *trp1* and *ura3* mutations are *leu2*-3,112, *trp1*-289 and *ura3*-52.

mutations in the arginine permease gene (*CAN*) that render the cells canavanine resistant. *UV responses of diploids*. Figure 1, panel D, illustrates the fact that *radH* is a recessive mutation : the heterozygous cells are as resistant as the *RADH* homozygotes. However, the *radH* homozygous diploids are highly UV sensitive. The kinetics of inactivation by low UV doses is close to that of G1 *radH* haploids. At higher doses, an inflexion of the

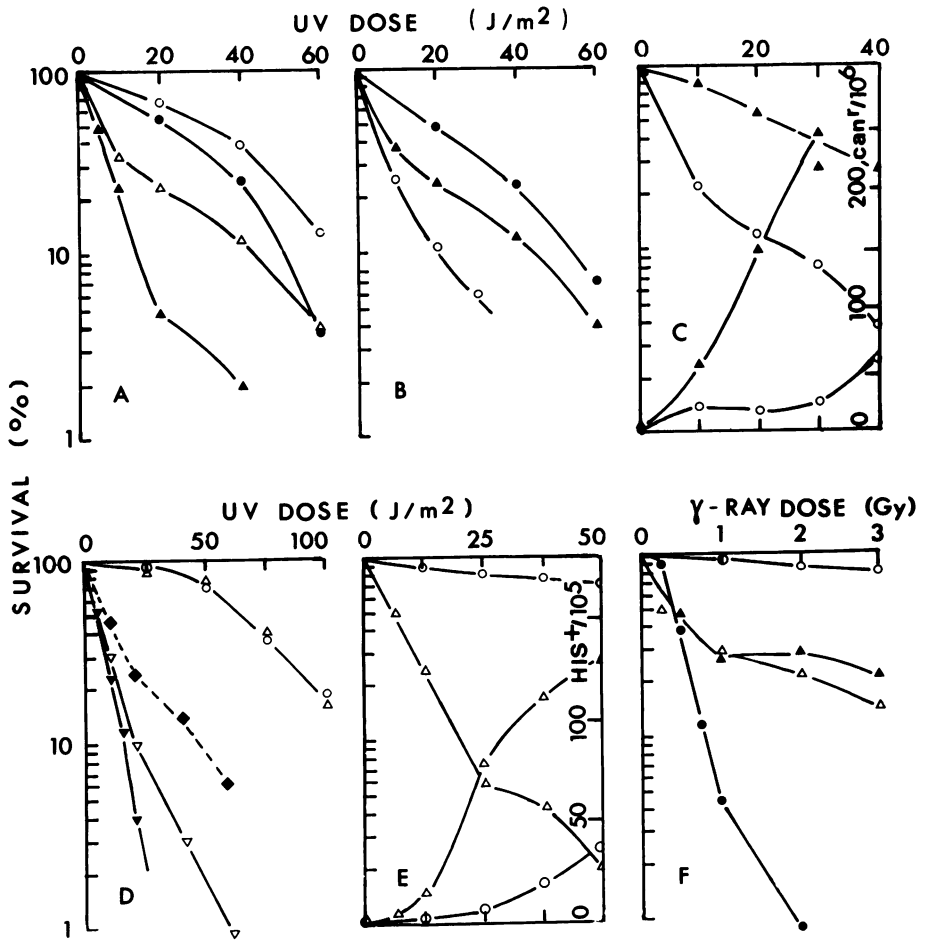

Figure 1: Radiobiological effect of the *radH* mutation. Panel A. UV survival of haploids. Circles, *RADH* (C6378); triangles, *radH* (FF18244). Open symbols, logarithmic (log.) phase cells. Close symbols, stationary (stat.) phase cells. Panel B. UV survival of the *rad50-radH* double mutant. ●, *rad50* (ZZ2); ▲, *radH* (FF18244); ○, *rad50-radH* (ZZ14). The cells were in log.phase. Panel C. UV-induction of forward mutations. Right ordinate, numbers of canavanine resistant mutant clones per 10^6 survivors. ▲, *RADH* (FF18266) ; ○, *radH* (FF18330). Panel D. UV survival of diploids treated in log.phase. ○, *RADH/RADH* (FF1848); △, *RADH/radH* (FF18404); ▽, *radH/radH* (FF18341). The *radH* (FF18244) survival ◆ is shown for comparison. ▼, *radH/radH* (FF18341) in stat. phase. Panel E. UV induction of gene conversion in diploids heteroallelic for *HIS7*. Right ordinate, numbers of His^+ colonies for 10^5 survivors. ○, *RADH/radH* (FF18700) ; △, *radH/radH* (FF18701). Panel F. Gamma-ray survival of haploids and diploids treated in log.phase. ▲, *RADH* (C6378) ; △, *radH* (FF18244) ; ○, *RADH/radH* (FF18404) ; ●, *radH/radH* (FF18341).

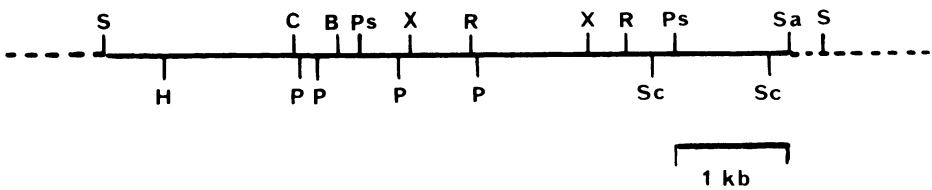

Figure 2: Restriction map of the *Sal*1-*Sau*3A insert that complement the *radH* mutation. B : *Bgl*II ; C : *Cla*1; H : *Hin*dIII; P : *Pvu*II ; Ps : *Pst*I ; R : *Eco*RV ; S : *Sal*I ; Sa : *Sau*3A ; Sc : *Sac*I ; X : *Xba*I.

curve is observed and may correspond to G2 cells. In any case, G2 diploids are more sensitive than G2 haploids.

Figure 1, panel E, shows that the UV-induction of gene convertants is higher in the homozygous *radH* cells than in the heterozygotes. This contrasts with the depressed induction of mutations seen in haploids.

Gamma-ray and methyl-methane sulfonate sensitivity. The results are shown in Figure 1, panel F. The gamma-ray sensitivity of *radH* haploids is comparable to that of wild-type cells. The characteristic resistance of G2 cells, due to recombinational repair of double-strand breaks (14) is not affected by the *radH* mutation. Diploids homozygous for the mutation are highly sensitive, and, as was the case after UV treatment, an inflexion that may correspond to G2 cells is observed on the survival curve.

A similar qualitative response was obtained with the radiomimetic MMS chemical. On YPD plates containing 0.012% of MMS, the *radH* homozygous diploids do not grow, contrarily to the heterozygous diploid and to the *RADH* or *radH* haploids. This property was used to clone the *RADH* gene by complementation.

Cloning of the RADH gene and its sequence

Cloning of the gene. The recessivity of the *radH* mutation and the high MMS sensitivity of the *radH* homozygous diploids allowed us to clone the *RADH* gene by complementation. *RadH* diploids (FF18341) were transformed with a centromeric YCp50 (ARS1-CEN4-URA3) bank (15). 1500 transformants were grown on master plates containing uracil-less medium and replica-plated on MMS (0.012%)-YPD medium. Four resistant clones were found, in which the MMS^R and the $URA3^+$ phenotypes mitotically cosegregated. The plasmids were extracted, amplified in *E. coli* and purified on CsCl gradients. A restriction map indicated that they all had a common region. One of the plasmids, called p14 was subcloned to delimit the complementing region. A restriction map of the p14-S subclone fully complementing the mutation, is shown on Figure 2.

That this insert contains the *RADH* gene is supported by the following experiment : the *Sal*1 fragment was subcloned into the non-centromeric YRp7 plasmid (ARS1-TRP1) (16). This YRp7-S plasmid was linearized by digestion with the *Sac*1 enzyme, which recognizes two sites inside the insert, in order to direct its integration into the homologous chromosomal region. Stable $TRP1^+$ transformants of the FF18-66 (*trp1-RADH*) strain were obtained and crossed with *radH-trp1* cells. In 24 complete tetrads originating from such diploids, the [radH] and $[TRP]^+$ phenotypes always segregated from each other, indicating that *TRP1* was integrated very close to the *RADH* locus. The proof that *RADH* is on the insert was later obtained by chromosomal disruption of the gene (data not shown).

Nucleotide sequence of the RADH gene. The sequence of the first 4640 nucleotides of the 6.6 kb *Sau*3A-*Sal*I fragment, containing the *RADH* coding region and its 5′ and 3′ flanking sequences is given in Figure 3. The *Sau*3A-*Cla*I fragment (Figure 2) was the shortest found to fully complement *radH* diploids.

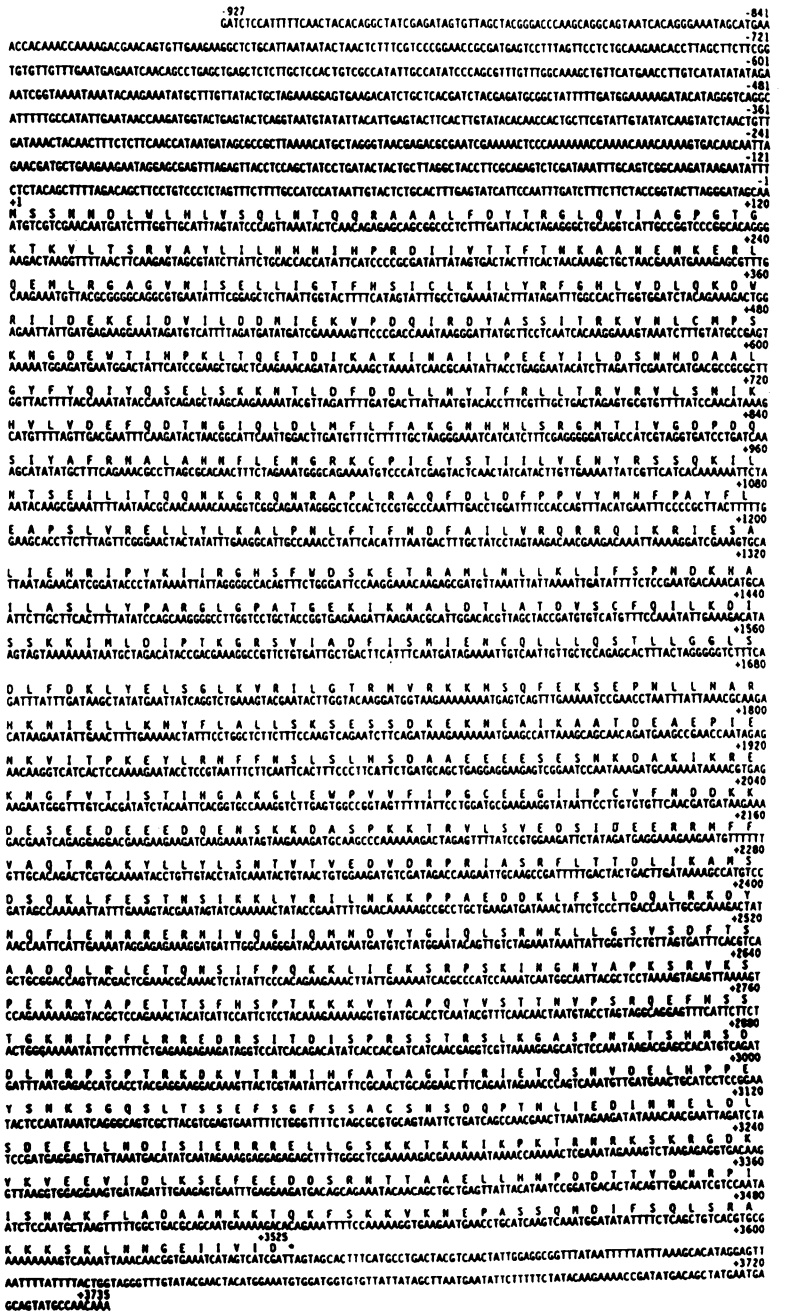

Figure 3: Nucleotide sequence of the *RADH* gene. The numbering is in relation to the first nucleotide of the ATG translation start codon, which is designated +1. The deduced aminoacid sequence of the RadH protein is given above the nucleotide sequence in the one-letter code.

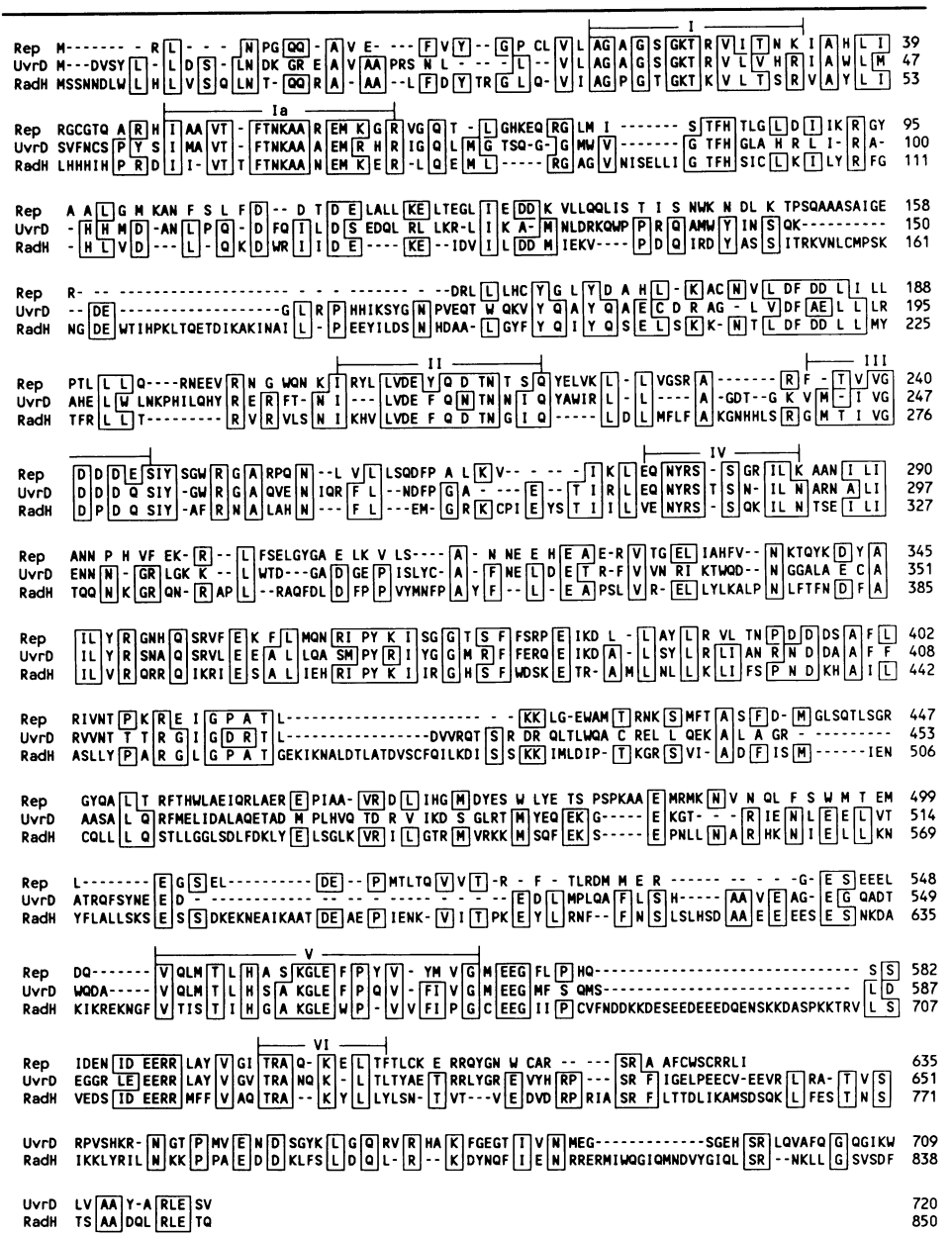

Figure 4: Comparison of the RadH, Rep and UvrD proteins. The identical residues are boxed. The seven consensus domains for DNA helicases are overlined and labelled according to Hogdman (22).

The *RADH* coding region contains a single open reading frame of 3525 nucleotides. The absence of the TACTAACCC sequence, thought to be a signal for RNA splicing (17) suggests that the *RADH* gene does not contain introns.

There is no apparent codon bias in favor of codons corresponding to major tRNA species

of yeast (18) and all the 61 codons are used. Many rare codons are found in the gene, suggesting that it is expressed at a low level.

5' and 3' flanking sequences. The *RADH* 5' and 3' flanking regions contain 59% and 64% A+T, respectively. The coding region contains 63% A+T. In the upstream region, a possible TATA box is found quite far from the ATG start codon (−610). In the 3' region, neither the consensus sequence AATAAA, which is believed to represent the signal for poly(A)$^+$ addition (19), nor the sequence TAG...TATGT...TTT, thought to be a transcription termination signal (20), are found.

The RadH protein has similarities with bacterial helicases. The *RADH* open reading frame encodes a protein of 1175 amino-acids with a predicted molecular weight of 134,172 daltons. It contains 14% acidic, 15% basic residues.

A characteristic ATP binding domain exists in positions 33−47. From position 222 to 243 four leucine residues are spaced seven residues apart. This may correspond to a 'leucine zipper' domain. Such structures have been proposed to be involved in the formation of homo- or hetero-dimers for DNA binding proteins (21). Thus, the RadH protein may interact, through its 'leucine zipper' with either itself or other proteins.

A computer search in the GenBank/EMBL data bank revealed significant homologies between the RadH protein and a number of DNA helicases. In fact, the seven consensus motifs found in DNA helicases (22) including the ATP binding site, are present in the RadH deduced protein. Besides these consensus regions the most significant homologies were found with the *E. coli* Rep (23) and UvrD (24) proteins (Figure 4). It is striking that the homologies with these bacterial proteins are much stronger than with the two known yeast helicases involved in DNA repair, the Rad3 (25,26) and the Pif1 (27) proteins, with which the homologies are essentially restricted to the consensus regions (data not shown).

It should be noted that the RadH protein is larger than the Rep, UvrD, Rad3 and Pif1 proteins (134 kDa compared to 68, 75, 87 and 97 kDa, respectively) and has a C-terminal region of about 325 amino-acids with no homology with any other known helicases.

DISCUSSION

The recessive *radH* mutation has the following effects: it sensitizes haploid cells treated in the G1 phase, but not haploids treated in the G2 mitotic phase, to the killing effect of UV. Homozygous diploids in G1 are almost as UV sensitive as G1 haploids, while, in G2 they are more sensitive than the corresponding haploids. After gamma-ray irradiation, haploids are not sensitized by the mutation, but diploids in G1 and to a lesser extent in G2, are sensitive. The UV-induction of mutations is depressed, contrarily to that of gene conversion which is increased.

This phenotype is different from that of all other radiation-sensitive mutants described in the literature, and was found to be the same in strains bearing a genomic deletion of the *RADH* gene. This deletion was obtained by the replacement of the *Eco*RV internal fragment (Figure 2) by the *Hpa*I-LEU2 fragment. A Southern analysis showed that the rearranged chromosomal structure was as expected (data not shown). The viability of the deleted strains indicates that the gene is not essential.

Other properties of the *radH* mutant (unpublished results) are the following : 1) The generation time of homozygous diploids is increased by a factor of 1.3 ; that of haploids is unaffected. 2) The rate of spontaneous mutation is unchanged ; the rate of spontaneous gene conversion is increased by a factor of about 10. 3) The sporulation and the frequency of meiotic gene conversion are unaffected but the spore viability is reduced by about 50%.

Our interpretation of the results concerning UV-lesions is the following : the *radH* mutation leads to a channelling into a recombination pathway of lesions engaged in the mutagenic repair process. The data in favor of the implication of *RADH* in mutagenic repair are : 1) the suppression by the mutation of the G2 UV-sensitivity of both *rad18* and *rad6* mutants (data not shown) which belong to the error-prone epistatic group of mutants ; 2) *the* decrease in induced mutagenesis seen in *radH* cells. The hypothesis of channelling into a recombinational pathway is based on the G1 sensitivity and G2 resistance of haploids: G1 cells cannot perform recombinational repair because of the lack of homologous molecules, while in G2, recombination between sister-chromatids can occur. The suppression of the G2 resistance of *radH* cells by the *rad50* mutation shows that recombination is indeed involved.

The *RADH* gene may be the *srs2* gene (28). Mutations in these two loci were found to map very close to each other. They partially restore the *rad18* and *rad6* UV sensitivity and are in this respect semi-dominant. The main differences are the absence of effects of the *SRS2* mutation in RAD^+ cells, and the absence of diploid sensitization by the homozygous *SRS2* mutation. The dominance of the mutations in the *rad18* diploids prevents doing complementation tests and it is not yet established whether or not *srs2* and *RADH* are one and the same gene.

The potentially mutagenic or recombinogenic structures are believed to be the same (29,30), i.e. a single-stranded region containing a pyrimidine dimer, resulting from the excision of a dimer in the opposite strand. Such a lesion could be processed by either the recombinogenic or the mutagenic system. In the *radH* mutant, the processing of those lesions engaged in mutagenic metabolism would be blocked and the resulting structure would be an unusual substrate for recombination proteins.

However, if recombination between sister-chromatids is successful, the events involving homologous chromosomes appear to be lethal : G1 diploids are as sensitive as G1 haploids, and the cells are deficient for induced mutagenesis but not recombinogenesis. It should be clear that, in our interpretation, the metabolism of the lesions engaged from the start in the recombination system is not affected by the *radH* mutation. The lethal recombination events are those initiated by the structures resulting from the deficiency in mutagenic repair.

In *S. cerevisiae*, two DNA helicases involved in DNA repair are known. The Rad3 protein is a non-essential helicase (31) involved in pyrimidine dimer excision. The nuclear *PIF1* gene encodes a putative DNA helicase involved in recombination, repair and stability of mitochondrial DNA (27). The comparison of the properties of the *rad3*, *pif1* and *radH* mutants strongly support the view that the 3 gene products are not functionally related. The RadH putative helicase appears to belong to the error-prone repair machinery and not to be involved in other repair processes, at least for UV lesions. It may act in association with the Rev3 protein, known to be the DNA polymerase involved in error-prone repair (32).

Besides the consensus regions found in DNA helicases, the RadH protein does not share strong homologies with the yeast Rad3 and Pif1 proteins but presents similarities with the Rep and UvrD helicases of *E. coli*. The properties of the *rep* and *UvrD* genes are however not reminiscent of those of *radH* cells. A characteristic of the *RADH* gene product is its higher Mr compared to those of other helicases and notably a carboxy-terminal region beyond the consensus domains containing no homology with other helicases.

The protein domains outside the consensus regions are likely to be involved in the in vivo specificity of the different helicases by allowing defined protein-protein interactions.

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