**XRS2, a DNA Repair Gene of Saccharomyces cerevisiae, Is Needed for Meiotic Recombination**

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Manuscript received March 27, 1992
Accepted for publication July 31, 1992

**ABSTRACT**

The XRS2 gene of *Saccharomyces cerevisiae* has been previously identified as a DNA repair gene. In this communication, we show that XRS2 also encodes an essential meiotic function. Spore inviability of xrs2 strains is rescued by a *spo13* mutation, but meiotic recombination (both gene conversion and crossing over) is highly depressed in *spo13 xrs2* diploids. The xrs2 mutation suppresses spore inviability of a *spo13 rad52* strain suggesting that XRS2 acts prior to RAD52 in the meiotic recombination pathway. In agreement with the genetic data, meiosis-specific double-strand breaks at the ARG4 meiotic recombination hotspot are not detected in xrs2 strains. Despite its effects on meiotic recombination, the xrs2 mutation does not prevent mitotic recombination events, including homologous integration of linear DNA, mating-type switching and radiation-induced gene conversion. Moreover, xrs2 strains display a mitotic hyper-rec phenotype. Haploid xrs2 cells fail to carry out G2-repair of gamma-induced lesions, whereas xrs2 diploids are able to perform some diploid-specific repair of these lesions. Meiotic and mitotic phenotypes of xrs2 cells are very similar to those of *rad50* cells suggesting that XRS2 is involved in homologous recombination in a way analogous to that of RAD50.

The XRS2 gene of *Saccharomyces cerevisiae* has been defined by mutations that make haploid and diploid cells highly sensitive to ionizing radiation. At the same time, xrs2 cells show only slightly increased sensitivity to UV light (Fedorova 1969; Suslova 1969; Suslova and Zakharov 1970; Zakharov, Suslova and Fedorova 1970). Analysis of properties of double mutant strains (Zelevnyakova et al. 1975; Suslova, Fedorova and Zelevnyakova 1975) allowed XRS2 to be assigned to the epistasis group of the RAD50-RAD57 genes known to be involved into a recombinational pathway of DNA repair [see Haynes and Kunz (1981) and Game (1983) for reviews]. Further analysis of xrs2 mutant diploids revealed that unlike mutations in the RAD52 gene, xrs2 mutations do not prevent mitotic recombination. On the contrary, xrs2 diploid cells show a hyper-rec phenotype, the frequency of spontaneous intragenic recombination being increased ten times compared to wild-type cells (Zakharov, Kassinova and Kovaltsova 1983). This effect of xrs2 mutations makes them similar to mutations in the RAD50 gene which also increase, rather than decrease, spontaneous mitotic recombination (Malone and Esposito 1981; Malone et al. 1990).

Besides their involvement in recombinational DNA repair, some genes of the epistasis group RAD50-RAD57 were shown to play a role in meiotic recombination and meiosis. Mutations in the RAD52 gene dramatically reduce intra- and intergenic meiotic recombination and produce inviable spores (Game et al. 1980; Prakash et al. 1980; Malone 1983; Borts, Lichtten and Haber 1986). Meiotic defects of rad52 strains are not suppressed in the presence of a *spo13* mutation, which eliminates the reductional meiotic division (Klapholz and Esposito 1980). The inability of rad52 mutants to be rescued by a *spo13* mutation was interpreted to mean that RAD52 acts after the initiation steps of meiotic recombination have been completed (Malone 1983; Malone et al. 1991). Mutations in the RAD50 gene also lead to greatly reduced sporulation and spore viability and to almost complete elimination of meiotic recombination (Game et al. 1980; Malone and Esposito 1981; Malone 1983; Borts, Lichtten and Haber 1986). However, in the presence of a *spo13* mutation, rad50 diploid strains sporulate well and produce viable spores (Malone and Esposito 1981; Malone 1983). It is generally believed that meiotic Rec" mutants producing viable spores in the presence of *spo13* represent a group of genes that function "early" in the meiotic recombination process, at the time of homolog pairing, formation of synaptonemal complex and initiation of strand exchange (Malone et al. 1991; Petes, Malone and Symington 1991). Consistent with and confirming this view are recent observations that two important events in yeast meiosis, occurrence of meiotic-specific double-strand breaks and formation of tripartite syn-
apotemonal complex (SC) structures, are blocked in rad50 deletion mutants (CAO, ALANI and KLECKNER 1990; ALANI, PADMORE and KLECKNER 1990). Up to now, RAD50 is the only known DNA repair gene involved in an “early” event(s) of yeast meiosis. Similarities in mitotic phenotypes between rad30 and xrs2 mutants suggest that XRS2 might well be another yeast DNA repair gene needed at an “early” stage(s) of meiotic recombination.

To test this hypothesis, we have undertaken a systematic study of the role of the XRS2 gene in meiotic and mitotic recombination. We were able to show that meiotic defects of xrs2 strains could be suppressed by a spo13 mutation and that xrs2 spo13 double mutant diploids showed depressed meiotic recombination. Meiosis-specific double-strand breaks at the ARG4 meiotic recombination hotspot are not detected in xrs2 deletion strains. At the same time, the xrs2 mutation increased spontaneous intra- and intergenic mitotic recombination and did not prevent radiation-induced gene conversion and mating-type switching. The whole body of evidence suggests that the XRS2 gene is involved in homologous recombination in a way similar to that of RAD50.

MATERIALS AND METHODS

Strains and plasmids: Yeast strains used to study properties of the xrs2-1 mutation are listed in Table 1. Additional strains from our laboratory collections were used to construct combinations of markers and for complementation and mating-type testing. In the course of strain construction, all strains containing the xrs2-1 mutation were backcrossed five times with Rad+ strains carrying the desired marker configuration. To minimize further effects due to strain differences, most experiments were carried out in two different, though related, genetic backgrounds.

Deletion/disruption alleles of XRS2, SP013, RAD50 and RAD52 genes were constructed by the one-step gene disruption method of ROTHSTEIN (1983). To disrupt XRS2, plasmid pEI40 containing a part of the XRS2 coding region substituted with a 2.0-kb HpaI-HpaI LEU2 fragment was used (E. IVANOV, unpublished results). pEI40 was digested with BamHI and HindIII and used to transform recipient strains to Leu+. pEI40 was digested with BamHI and EcoRI and used to transform recipient strains to Ura+.

Recombination tests and sporulation: All diploid strains were freshly constructed before each experiment. To assay meiotic intragenic recombination at the HIS7 locus, six independent colonies for each strain grown on YEPD plates were inoculated into 5 ml of liquid YEPD and incubated for 24 hr. One hundred-fold dilutions were then made into 5 ml of presporulation medium (YPA) and incubated until the cell density reached 2 × 10^7 cell/ml. One half of the cell suspensions was then spun down, washed twice with saline (0.9% NaCl) and plated on SC-HIS omission plates to estimate mitotic levels of His+ prototrophs and on YEPD plates to determine the number of viable cells. The other half of the cell suspensions was washed once with saline and once with liquid sporulation medium (SM), resuspended in 5 ml of SM at 1–2 × 10^7 cell/ml and incubated for 3–4 days with vigorous shaking. Appropriate dilutions were then plated onto SC-HIS and YEPD plates to measure mitotic levels of recombination.

To assay meiotic-specific double-strand breaks at the ARG4, a modification of this protocol was used. A single colony of a SKI diploid was inoculated into 5 ml of liquid YEPD and incubated for 8–10 hr. The cells were then transferred into 100 ml of YPA and grown overnight to the cell density 1–2 × 10^7 cell/ml. Cells were harvested, washed with SM, resuspended in 100 ml of SM, and incubated with vigorous shaking. Aliquots (10–25 ml) of sporulating cultures were removed from SM and fixed in ethanol at −20°C.

Rates of spontaneous mitotic recombination were determined in a fluctuation test by the median method of LEA and COULSON (1948). Twenty cultures for each strain were initiated from 50–70 cells and grown to saturation. Rates were calculated by the following equation (DRAKE 1970):

\[
\text{Rate} = \frac{0.4343 \times \text{median frequency}}{\log(\text{final cell}) - \log(\text{initial cell})}
\]

To determine induced frequencies of intragenic recombination, exponentially growing cells were washed and resuspended in saline and irradiated either with gamma rays (60Co, dose rate 1.36 krad/min) or with UV light (254 nm, dose rate 1 J/m^2/sec). Dilutions of irradiated cell suspensions were plated on SC-HIS plates to determine frequencies of recombination and on YEPD plates to determine cell viability. All irradiation experiments were repeated at least twice.

Spontaneous mitotic segregation of markers on chromosome V was measured as follows. Rates of appearance of Can+ colonies were determined in a fluctuation test essentially as described above, except that cultures were grown to a cell density of 5 × 10^7/ml and after washing twice in saline, were plated on SC plates supplemented with 60 mg/liter of canavanine sulfate (Sigma). To test phenotypes of Can+ segregants, a single colony for each strain was suspended in sterile water and the suspensions were plated on YEPD medium in order to obtain 60–80 colonies per plate. After 2 days the colonies were replicated onto SC + CAN plates and after 2 more days Can+ papillae appeared.
### TABLE 1

#### Yeast strains

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<th>Genotype</th>
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<td>a his7-1 leu2-3,112 lys2-1 URA3-52 XRS2 rad50::URA3</td>
</tr>
</tbody>
</table>
from the colony prints. To ensure independent origin of every Can⁴ clone, only one papilla was taken from a colony print. The Can⁴ papillae were picked up onto SC + CAN master plates and replicated the next day onto SC-URA, SC-ISO and SC-HIS plates.

DNA manipulations: Escherichia coli strain TG1 (supE hsdSΔ5 thi Δ(lac-proAB) F′(traD36 proAB⁺ lacIq lacZΔM15)) was used for plasmid propagation. Plasmid DNA was isolated by standard procedures (Maniatis, Fritsch and Sambrook 1982). Restriction enzymes were purchased from New England Biolabs and used according to the manufacturer’s specifications. Yeast transformations were performed by the lithium acetate method (Ito et al. 1983).

Detection of meiosis-specific double-strand breaks at the ARG4 was carried out as described by Sun et al. (1989). Yeast DNA was purified essentially as in Cao, Alaniz and Kleckner (1990) with minor modifications proposed to us by B. de Massy. Total DNA was digested with BglII and run on 0.7% agarose gels. DNA were transferred to nylon membrane (Hybond-N+, Amersham) in 0.4 M NaOH for 3 hr. ³²P-labeled hybridization probes were prepared by the random primer method (Feinberg and Vogelstein, 1984) using the Applied Genie kit. Hybridization and washing of filters was carried out according to Church and Gilbert (1984). Blots were exposed to XAR-5 films in the presence of intensifying screen for up to several days.

RESULTS

xrs2-1 causes meiotic defects that can be rescued by spo13: Table 2A shows that the xrs2-1 mutation dramatically reduces sporulation of the diploid strain E1303. Five more xrs2-1/xrs2-1 diploid strains constructed in the course of this study were tested for sporulation and showed 1–10% sporulation. In total, 60 tetrads derived from four of these strains, including E1303, were dissected and no viable spores were obtained. This means that the spore viability of xrs2-1/xrs2-1 diploids is less than 0.5%. To determine whether the low spore viability can be rescued by a spo13 mutation, double mutant strains have been constructed and shown to sporulate well and to produce highly viable ascospores (Table 2B). Therefore, meiotic inviability caused by the xrs2-1 mutation cannot be circumvented by the absence of the reductional meiotic division.

xrs2-1 reduces meiotic recombination in spo13 diploids: Spore inviability of xrs2-1/xrs2-1 diploids suggests that the xrs2-1 mutation affects meiotic recombination. The ability of xrs2-1 spo13 double mutants to produce viable ascospores allowed us to address this question. The effect of xrs2-1 on meiotic intragenic recombination was assayed by using a pair of his7 heteroalleles. To establish the amount of recombination occurring before meiosis, the level of mitotic recombination was also determined in the same diploids. Table 3 indicates that wild-type diploids E1309 and E1310 show typical meiotic increases in spore inviability of xrs2-1/xrs2-1 strains. The xrs2-1 mutation did not affect meiotic inviability caused by the spo13-1 mutation.
TABLE 2

Sporulation and spore viability in xrs2 diploid strains

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Strain</th>
<th>Percent sporulation</th>
<th>Percent spore viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) SPO13 strains&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>XRS2</td>
<td>E1302</td>
<td>83</td>
<td>87</td>
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<tr>
<td>xrs2-1</td>
<td>E1304</td>
<td>77</td>
<td>79</td>
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<tr>
<td>xrs2-1</td>
<td>E1303</td>
<td>10</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>B) spo13Δ strains&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spo13Δ XRS2</td>
<td>E1309</td>
<td>53</td>
<td>62</td>
</tr>
<tr>
<td>spo13Δ xrs2-1</td>
<td>E1310</td>
<td>49</td>
<td>54</td>
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<tr>
<td>spo13Δ xrs2-1</td>
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<td>84</td>
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<tr>
<td>spo13Δ XRS2 rad52Δ</td>
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<td>51</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.6</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
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<td>1.5</td>
<td>&lt;2.5</td>
</tr>
<tr>
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<td>E1314</td>
<td>33</td>
<td>57</td>
</tr>
<tr>
<td>spo13Δ xrs2-1 rad52Δ</td>
<td>E1318</td>
<td>57</td>
<td>85</td>
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</table>

<sup>a</sup> Included in Table 2A are only those strains used to assay spontaneous and induced mitotic recombination (see Table 5 and Figures 2 and 3). Percent sporulation was calculated by counting 200 cells, values being the averages from at least two colonies. Spore viability was determined by dissecting about 60 tetrads each for strains E1302 and E1304 and 15 tetrads for strain E1305 (see also text).

<sup>b</sup> Percent sporulation was calculated as for SPO13 strains. Spore viability was determined by dissecting 20 dyads for strain E1315 and about 100 dyads each for strains E1309, E1310, E1311, E1314 and E1318.

<sup>c</sup> ND, not determined.

To determine whether the xrs2-1 mutation reduces meiotic crossing over as well, we constructed a series of strains homozygous for the spo13-1 mutation, dissected dyads from sporulated cultures and tested recombination in four intervals on three different chromosomes. The dissection data presented in Table 4 clearly indicate that the xrs2-1 mutation essentially eliminates crossing over: as a matter of fact, only one dyad interpreted as recombinational was observed. The precise estimation of the extent to which xrs2-1 reduces meiotic crossing over was hampered by the high level of genetic instability seen in our spo13-1 diploid strains. Indeed, only a minority of dyads preserved the parental (i.e., nonrecombined) configuration of the leu1 marker in wild-type spo13-1 diploids. Unusually high levels of recombination in the centromere VI-LEU1 interval in spo13 diploids have been described (KLAPHOLZ and ESPOSITO 1980; MALONE and ESPOSITO 1981). This can reflect not only an unexpectedly high level of crossing over prior to the equational division per se, but also a higher probability of reducational segregation as well as different types of aberrant segregation (for details, see KLAPHOLZ and ESPOSITO 1980). 1<sup>+</sup>1<sup>−</sup> marker segregation may also indicate gene conversion. Gene conversion during sporulation of spo13 strains has been demonstrated for several markers, including leu1, and appears to be increased (KLAPHOLZ and ESPOSITO 1980). Therefore, when extrapolating from the wild type to the xrs2-1/xrs2-1 strain, we did not take into account leu1 dissection data. Extrapolation made on the basis of the rest of the dissection data would predict approximately 150 recombinant dyads for the xrs2-1/xrs2-1 strain. Thus, crossing over is reduced more than 100-fold by the xrs2-1 mutation.

The amount of aberrant segregation is also reduced by the xrs2-1 mutation. Combined with higher levels of sporulation and spore viability seen in a xrs2-1 spo13 strain as compared with a XRS2 spo13 strain (Table 2B), our data extend observations made by others that decreased meiotic recombination in spo13 cells generally improves the yield of viable meiotic products (MALONE and ESPOSITO 1981; KLAPHOLZ,

TABLE 3

Effect of xrs2-1 mutation on meiotic intragenic recombination at the HIS7 gene in spo13Δ diploids

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Strain</th>
<th>Mitotic</th>
<th>Meiotic</th>
</tr>
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<tr>
<td>spo13Δ XRS2</td>
<td>E1309</td>
<td>9.9 ± 1.0</td>
<td>1480 ± 141</td>
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<tr>
<td>spo13Δ xrs2-1</td>
<td>E1310</td>
<td>6.4 ± 1.0</td>
<td>1242 ± 94</td>
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<td>spo13Δ xrs2-1</td>
<td>E1311</td>
<td>69 ± 15</td>
<td>68 ± 7</td>
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<td>E1314</td>
<td>1.7 ± 0.3</td>
<td>7.3 ± 4.1</td>
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</table>

<sup>a</sup> Six independent cultures were examined for each strain. The means with standard errors are presented.
Produced sporulation and spore viability due to the mutation on meiotic recombination in the strain. As shown in Table 2B, however, triple mutant diploids *spo13* *xrs2*-*I* *rad52* were able to sporulate and to produce viable spores (MALONE 1983). To test whether *xrs2*-*I* behaves in this respect similar to *rad50* mutations, the triple mutant diploid strain E1314 (*spo13* *xrs2*-*2* *rad52*) was constructed. As shown in Table 2B, this strain was able to sporulate and produced viable spores although not so effectively as a strain heterozygous for *rad52* (E1318). This effect of *xrs2*-*I* is obviously recessive because it is absent in a strain heterozygous for *xrs2*-*1* (E1315). As expected, the triple mutant diploid E1314 was deficient in intragenic meiotic recombination (Table 3) as well as in meiotic crossing over between MAT and centromere III (data not shown). Therefore, the *xrs2*-*1* mutation can rescue the meiotic inviability of *rad52* in the presence of *spo13*.

**Meiosis-specific double-strand breaks at the ARG4 region are not detected in *xrs2* mutants:** Meiosis-specific double-strand breaks (DSBs) are essential early intermediates in the meiotic recombination process. DSBs were shown to occur at high frequency at the initiation site for meiotic gene conversion in the *ARG4* and in the 5′-region of the proximal DED81 gene (SUN et al. 1989) as well as in a hotspot for meiotic reciprocal recombination on the chromosome III (CAO, ALANI and KLECKNER 1990). Effects of the *xrs2*-*1* mutation on meiotic recombination suggest that *XRS2* functions at an "early" stage in the meiotic recombination pathway. This led us to analyse the occurrence of meiosis-specific DSBs at the *ARG4* region in SK1 derived strains bearing a deletion allele of the *XRS2* gene (E. IVANOV, unpublished results).

DSBs appearing at the *ARG4* region are most abundant between three and five hours after transfer of cells to sporulation medium (SUN et al. 1989). In our experiments, strains E1413 (*XRS2/XRS2*) and E1414 (*xrs2::LEU2/xrs2::LEU2*) were induced to sporulation. Total DNA was purified from cells prior to (0 hr) or 4.5 hr after entry into meiosis, digested with *Bgl*II and analysed by Southern blots as described in MATERIALS AND METHODS. DNA fragments corresponding to DSBs at site I (3.5 kb) and site II (1.5 kb) were observed in the wild-type strain after 4.5 hr of sporulation (Figure 1). These fragments were not detected in the *xrs2* strain. We cannot explain the appearance of the 1.3-kb fragment, however, the latter is only present in strain E1413 and is obviously meiosis-non-specific. One can exclude formally that meiotic DSBs occur in the *xrs2* strain but with altered kinetics, i.e., before or after 4.5 hr of sporulation. We consider this possibility to be unlikely for the following reason. Although ascospore formation is greatly reduced in strain E1414, first rare asci appear at the same time.
xrs2-1 mutation increases the frequency of spontaneous mitotic recombination at the HIS7 gene in a spo13 diploid (Table 3). To see whether this is generally true for mitotic cells, we determined rates of intragenic recombination in spo13 strains using the same pair of his7 heteroalleles. Results of the fluctuation test presented in Table 5 show that xrs2-1 causes about a 10-fold increase in recombination rates. Essentially the same, 12-fold, increase in recombination rates was observed in a xrs2 mutant diploid bearing a pair of arg4 heteroalleles (E. Ivanov, unpublished results). We also studied the effect of the xrs2-1 mutation on induced intragenic recombination in the same strains. The results demonstrate that xrs2-1 does not dramatically affect induction of recombination by two agents, gamma rays and UV light, frequencies of His+ prototrophs being similar to those of the wild-type strain (Figure 2) and only moderately decreased after UV light treatment (Figure 3). We conclude that the xrs2-1 mutation increases spontaneous mitotic intragenic recombination and does not prevent its induction by radiation.

**xrs2-1 increases spontaneous mitotic crossing over:** To determine whether the xrs2-1 mutation affects spontaneous mitotic intergenic recombination, we constructed a series of diploid strains heterozygous for the recessive drug-resistance marker Can^R (can1), on the chromosome V. The chromosome that carried the Can^R marker was also marked on the same (ura3) or the other (iso1 and his1) arm. Diploids may become resistant to canavanine either by mitotic recombination or by loss of the chromosome carrying the dominant sensitive (can1) allele. In the latter case, recessive markers on the opposite side of the centromere relative to the CAN1 would be expressed. First, rates of appearance of Can^R colonies were determined in a fluctuation test and were shown to be increased 15–20-fold for a xrs2-1/xrs2-1 strain (Table 6). Further, approximately 400 drug-resistant colonies for each strain were picked and tested for the expression of recessive markers on both arms of chromosome V.

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Results shown in Table 6 clearly indicate that xrs2-1 mutation does not cause chromosome loss. Only four Can^R colonies can be interpreted to result from chromosome loss (although they might well have appeared as the result of double crossover events), but the frequency of their appearance is too small to be statistically significant ($\chi^2 = 0.78; P < 0.25$). Therefore, xrs2-1 increases spontaneous mitotic recombination without concomitant chromosome loss.

Can^R colonies obtained in this experiment could arise either by gene conversion or by crossing over. In the latter case, crossing over between the CAN1 locus and the centromere should lead to a certain amount of homozygotization for the ura3 marker. In wild-type diploids, approximately 20% of the Can^R clones were also Ura^- (Table 6). In the mutant strain significantly fewer Can^R segregants were homzygous for the ura3 marker ($\chi^2 = 20.0; P < 0.005$). This might mean that the increased rate of Can^R colonies seen in the xrs2-1/xrs2-1 diploid was due mainly to enhanced gene conversion. The calculated rate of crossing over between the centromere and URA3 locus for strain E1287 is equal to $\left(50 \times 10^{-5}\right) \times 0.08 = 4.0 \times 10^{-5}$, whereas the same values for strain E1291 equals $\left(3.5 \times 10^{-5}\right) \times 0.23 = 8.1 \times 10^{-6}$ and for strain E1288, $\left(2.2 \times 10^{-5}\right) \times 0.20 = 4.4 \times 10^{-6}$. Thus, the $xrs2-1$ mutation does increase spontaneous mitotic crossing over, at least on chromosome $V$.

*xrs2-1 does not prevent homothallic mating-type switching*: Mating-type switching in *S. cerevisiae* is a highly efficient mitotic gene conversion event initiated by a double-strand break in the MAT locus made by the HO endonuclease. It appears that MAT switching requires essentially the same gene functions as do other mitotic recombination events, including the RAD52 gene (reviewed in White and Haber 1990). It was shown that rad52 mutations prevent homothallic MAT conversions and that HO rad52 strains are inviable (Malone and Esposito 1980; Weiffenbach and Haber 1981).

To determine the effect of the $xrs2-1$ mutation on mating-type switching, two diploids (E1299 and E1300) have been constructed, each heterozygous for $xrs2-1$ and HO. Analysis of the segregation of mating type in these diploids showed the pattern expected for ho/HO diploids, i.e., 2 maters:2 nonmaters (Table 7A). Further analysis of the mating phenotypes of $xrs2-1$ and XRS2 spore clones revealed essentially the same (and close to the expected 1:1:2 distribution of MATa, MATa, and N phenotypes (Table 7B). This clearly demonstrates that $xrs2-1$ does not prevent
mating-type switching, and that the expression of the HO gene does not lead to inviability of xrs2 spores.

xrs2-1 prevents G2- but not diploid-specific repair of γ-ray-induced lesions: The XRS2 gene has been initially defined by mutations leading to sensitivity to ionizing radiation (see Introduction). Figure 4 shows that the increased sensitivity of a haploid xrs2 strain to γ-rays can be completely accounted for by the elimination of a radioresistant "tail" corresponding to a fraction of budding (G2) cells. In this respect, xrs2-1 responds to γ-irradiation similarly to isogenic rad50 and rad52 strains (Figure 4). Figure 4 also shows that sensitivity of double xrs2-1 rad50 and xrs2-1 rad52 mutants is essentially the same as for corresponding single mutants implying epistatic interactions between XRS2 and RAD50 and XRS2 and RAD52.

Figure 5 shows responses to γ-irradiation of homozygous diploid strains. Comparison of Figures 4 and 5 demonstrates that the diploid wild-type strain is more resistant to γ-rays than the wild-type haploid, an effect known as diploid-specific repair (SaeK, Machida and Nakai 1980). Homozygous xrs2/xrs2, rad50/rad50 and xrs2/xrs2 rad50/rad50 diploids, although more sensitive than wild-type strain, nevertheless display diploid-specific increase in resistance. In sharp contrast, this effect is completely absent in the case of a rad52/rad52 diploid, where both haploid and diploid cells show essentially the same sensitivity to γ-rays (Figure 5). We concluded, therefore, that the xrs2-1 mutation completely blocks G2-repair but
only partially affects diploid-specific repair of \( \gamma \)-ray-induced DNA damages.

**xrs2-1 is not an allele of the CDC40 gene:** The XRS2 gene has been mapped to the right arm of chromosome IV, close to CDC40 (Kova\v{z}tzo\v{a} et al. 1990) raising a possibility that the xrs2-1 is an allele of this gene known to be involved in DNA repair (Kassir et al. 1985; Kupiec and Simchen 1986). To test this possibility, diploid strain E1254 heterozygous for xrs2-1 and cdc40-1 has been constructed. Analysis of its properties has shown that all mutant phenotypes due to both mutations were mutually complemented. Genetic analysis indicated that xrs2 and cdc40 are 2 cM apart (data not shown). Therefore, XRS2 and CDC40 are different genes.

**DISCUSSION**

The XRS2 gene was initially identified as a gene involved in repair of DNA damage induced by ionizing radiation (Fedorova 1969; Suslova 1969; Suslova and Zakharov 1970; Zakharov, Suslova and Fedorova 1970). In this communication, we report on radiation responses and recombination properties of xrs2 cells and present evidence that XRS2 encodes an essential meiotic function. Indeed, diploids homozygous for the xrs2-1 mutation sporulate poorly and produce completely inviable spores (Table 2). Poor sporulation and ascospore inviability of xrs2 strains can be rescued in the presence of a spo13 mutation which eliminates the first (reductional) meiotic division and hence bypasses the need of recombination for the proper segregation of chromosomes in meiosis (Klapolz and Esposito 1980). Analysis of spo13 xrs2 double mutant diploids reveals that the xrs2-1 mutation dramatically reduces meiotic recombination, both gene conversion at the HIS7 (Table 3) as well as crossing-over in intervals spanning a total of 128 cM on three different chromosomes (Table 4).

A number of yeast genes have been described, mutations of which decrease meiotic recombination, sporulation and spore viability, the two latter defects being suppressed by a spo13 mutation: RAD50 (Malone and Esposito 1981; Alani, Padmore and Kleckner 1990; SPO11 (Klapolz, Wadell and Esposito 1985), RED1 (Rockmill and Roeder 1988, 1996), HOP1 (Hollinsworth and Byers 1989), MEH4 (Menees and Roeder 1989), MER1 (Engbrecht and Roeder 1989), MER2/REC107 (Engbrecht, Hirsch and Roeder 1990; Cool and Malone 1990), REC102 (Malone et al. 1991; Bhargava, Engbrecht and Roeder 1992), MEX1/MRE4 (Rockmill and Roeder 1991; Leem and Ogawa 1992). The ability of mutations leading to reduced sporulation and spore viability to be rescued by a spo13 mutation is generally interpreted to mean that the corresponding genes function at “early” stages in the meiotic recombination process (Malone et al. 1991; Petes, Malone and Symington 1991). Properties of spo13 xrs2 double mutants suggest that XRS2 represents a novel member of the group of “early” meiotic Rec genes.

If XRS2 is indeed involved in meiotic recombination, we can ask whether it belongs to the RAD52-dependent pathway. Meiotic defects of rad52 diploids are not suppressed in the presence of a spo13 mutation (Malone and Esposito 1981; Malone 1983) suggesting that RAD52 acts at later stages of the meiotic recombination process. However, addition to spo13 rad52 diploids of a mutation in some of the “early” Rec genes improves sporulation and spore viability (Malone 1983; Engbrecht and Roeder 1989; Menees and Roeder 1989; Malone et al. 1991; Bhargava, Engbrecht and Roeder 1992). If xrs2 mutation confers a block in recombination prior to the RAD52 step, sporulation and spore viability should be rescued in spo13 xrs2 rad52 diploids. We observed that this was indeed the case (Table 2). In a similar way, the xrs2 mutation was able to improve low sporulation and spore viability of a spo13 rad51 strain (F. Fabre and E. Ivanov, unpublished results). Mutations in the HOP1 and RED1 genes do not seem to be able to rescue meiotic defects of spo13 rad52 strains (Malone et al. 1991). HOP1 and RED1 are thought to be involved primarily in the chromosome pairing pathway in meiosis (Hollinsworth and Byers 1989; Hollingsworth, Goetsch and Byers 1990; Rockmill and Roeder 1988, 1990). The ability of the...
**XRS2 and Meiotic Recombination**

xrs2-1 mutation to rescue meiotic defects of spo13 rad52 diploids suggests that XRS2 is involved primarily in the meiotic recombination, rather than in chromosome pairing, and that it acts prior to RAD52 in the recombination pathway.

Recent observations have clearly demonstrated that meiotic recombination in yeast is initiated by meiosis-specific double-strand breaks (DSBs) followed by their processing into extensive 3'-overhanging, single-stranded tails (Sun et al. 1989; Cao, Alani and Kleckner 1990; Sun, Treco and Szostak 1991). It was also shown that the formation of these meiotic DSBs is prevented or essentially reduced by null mutations in genes belonging to the group of "early" meiotic Rec genes: RAD50, SPO11 (Alani, Padmore and Kleckner 1990; Cao, Alani and Kleckner 1990), and MRE4 (Leem and Ogawa 1992). On the contrary, mutations in "late" meiotic Rec genes such as RAD51 (Shinohara, Ogawa and Ogawa 1992) and RAD52 (A. Shinohara and T. Ogawa, personal communication cited in Leem and Ogawa 1992) lead to the accumulation of anomalously degraded forms of DSBs. Our observations that meiosis-specific DSBs at the ARG4 hotspot for meiotic gene conversion are not detected in xrs2 deletion strains (Figure 1) are in good agreement with genetic data implying that XRS2 belongs to the group of "early" meiotic Rec genes. Our results provide also an additional support to the view that "early" meiotic Rec genes act before or at the time of the formation of meiotic DSBs.

None of the "early" meiotic Rec genes, except RAD50, appear to have mitotic functions since the corresponding mutants do not affect mitotic recombination and/or DNA repair. So far, RAD50 is the only gene of this group shown to play a role in mitosis. Our results demonstrate that it is also true for XR52. Homozygous xrs2 diploids are more sensitive to gamma-irradiation than wild-type cells but are still more resistant to the treatment than corresponding haploids, demonstrating a partially active diploid-specific repair (Figures 4 and 5). This effect is also prominent in wild-type diploids and homozygous rad50 diploids (see also SaeKI, Machida and Nakai 1980) but is completely absent in rad52 diploid strains known to be defective in DSB repair (Ho 1975; Resnick and Martin 1976). We interpret these data to indicate that xrs2 cells are able to repair some of the radiation-induced DSBs. It was reported recently that rad50 cells can repair DSBs introduced by HO endonuclease, albeit with limited efficiency (Sugawara and Haber 1992). The latter result would explain both the increased radiation sensitivity of rad50 diploid cells compared with wild-type cells as well as their ability to perform some diploid-specific repair.

Despite the ability of diploid cells to perform some diploid-specific repair, haploid xrs2 cells fail to carry out G2-repair of γ-ray-induced DNA lesions; the same is true also for haploid rad50 cells (Figure 4). The inability to perform G2-repair suggests a defect in sister chromatid exchange (SCE). It has been shown that the rad50 mutation completely blocks SCE in meiosis in nonribosomal DNA (Game et al. 1989; Gottlieb, Wagstaff and Esposito 1989; Sun, Dawson and Szostak 1991). Whether the rad50 mutation also influences mitotic SCE, is less clear. Although no differences were observed between rad50 and RAD50 strains in the frequency of intrachromosomal recombination using duplicated his4 genes, analysis of recombination products was not performed in this work (Gottlieb, Wagstaff and Esposito 1989). It cannot be excluded that a decrease or even absence of SCE was masked by a concomitant increase in the yield of other events, reciprocal intrastrand exchange and gene conversion. Data on the influence of xrs2 and rad50 mutations on radiation-induced SCE are not yet available. We hypothesize that both xrs2 and rad50 haploid cells are specifically deficient in radiation-induced SCE. That would explain the inability of the cells to perform G2-repair after gamma-irradiation.

As discussed above, XR52 is required for meiotic recombination. At the same time, mitotic recombination events appear to proceed normally in xrs2 mutant strains. In the course of this work, a number of gene disruptions (SPO13, RAD50, RAD52) were made in xrs2 strains by homologous integration of linear DNA suggesting that XR52 is not necessary for this process. Of course, xrs2 deletion alleles should be analyzed to answer this question definitively. The xrs2 mutation does not also seem to affect another mitotic recombination process - mating-type switching (Table 7), implying that xrs2 cells are able to repair DSB introduced by HO endonuclease in the MAT locus. rad50 mutants have also been reported to perform homologous integration with linear plasmids (Alani, Padmore and Kleckner 1990) as well as MAT switching (Malone 1983), whereas rad52 strains are deficient in both of these processes (Malone and Esposito 1980; Orr-Weaver, Szostak and Rothstein 1981).

It has been shown that the xrs2 mutation increased UV-induced gene conversion at the Ade2 locus and decreased it after gamma-irradiation (Zakharyov, Kassinova and Kovaltzova 1983). In our experiments, when using a pair of his7 heteroalleles, no effect of the xrs2-I mutation on gamma-induced intragenic recombination was observed (Figure 2) and a moderate decrease was apparent after UV-induction (Figure 3). There are also conflicting reports on radiation-induced intragenic recombination in rad50 strains. Hunnable and Cox (1971, cited in Malone and Esposito 1980) reported that UV-induced gene conversion was not reduced by rad50-I, whereas Kupiec and Simchen (1984) observed complete elmi-
nation by rad50-1 of UV-induced gene conversion at the ADE2 and LYS2 loci. In turn, SAEKI, MACHIDA and NAKAI (1980) showed that rad50-1 mutation only slightly decreased intragenic recombination at the LEU1 gene induced by γ-irradiation. In our hands, a strain bearing a deletion allele of RAD50 and a pair of his7 heteroalleles showed no effect on intragenic recombination after UV-irradiation and no more than a twofold decrease in recombination after γ-irradiation (F. FABRE, unpublished results). We think that these contradicting data reflect the fact that both genes, XRS2 and RAD50, are dispensable for radiation-induced mitotic gene conversion, the particular results being dependent on the pair of heteroalleles used and/or experimental conditions.

The xrs2-1 mutation displays a mitotic hyper-rec phenotype. Previously, spontaneous intragenic recombination at the ADE2 locus was shown to be increased in xrs2 diploid cells (ZAKHAROV, KASSINOVA and KOVALTOVA 1983). We confirmed this observation by using a pair of heteroalleles at the HIS7 gene; in our experiments, rates of spontaneous recombination were approximately ten times higher in xrs2 diploid as compared with wild-type and heterozygous diploids (Table 5). Essentially the same results have been obtained when spontaneous recombination between a pair of arg4 heteroalleles were tested (E. IVANOV, unpublished results). Using strains with heterozygous markers of chromosome V we showed that xrs2 mutation increased spontaneous crossing over as well (Table 6). In rad50 cells, both spontaneous mitotic gene conversion and crossing over are also increased (MALONE and ESPOSITO 1981; MALONE et al. 1990; GOTTLIEB, WAGSTAFF and ESPOSITO 1989; ALANI, PADMORE and KLECKNER 1990). The hyper-rec phenotype of xrs2 and rad50 mutants could be a consequence of their defect in G2-repair. This defect is evidenced by the lack of G2-resistance in γ-irradiated haploid cells whereas diploids are able to perform some diploid-specific repair and are obviously recombination-proficient. It is known that both spontaneous (WILDENBERG 1970; ESPOSITO 1978) as well as radiation-induced (FABRE 1978; FABRE, BOULET and ROMAN 1984; ROMAN and RUIZINSKI 1990) recombination events occur primarily in the G1 phase of the cell cycle. We speculate that both XRS2 and RAD50 gene products are required for G2-repair of spontaneous recombinogenic lesions. Alternatively, these recombinogenic lesions could be produced in G2 due to the inability of xrs2 and rad50 mutant cells to perform some G2-specific functions. In any case, lesions would accumulate and if passing through mitosis would lead to recombination events in the following G1. A similar explanation of mitotic hyper-rec phenotype of rad50 mutants has been proposed recently by RAYMOND (1990).

In summary, the XRS2 gene previously known to be involved in DNA repair is needed also for meiotic recombination. XRS2 belongs to a group of "early" meiotic Rec genes and acts prior to RAD52 in the meiotic recombination pathway. xrs2 mutations do not prevent mitotic recombination; moreover, xrs2 cells display spontaneous hyper-rec phenotype. Meiotic and mitotic properties of xrs2 cells are very close to those of rad50 cells suggesting that XRS2 is involved in homologous recombination in a way similar to that of RAD50.

We thank A. ADJIRI, Y. KASSIR, B. DE MASSY, W. RAYMOND and D. SCHILD for providing strains and plasmids. We thank B. DE MASSY for advice on DNA purification and C. WHITE for help with Southern analysis. We are grateful to A. ABOUSSERHRA, A. ADJIRI, R. CHANTET, M. HEUDE, B. DE MASSY AND C. WHITE for helpful discussions. We thank C. WHITE and M. HEUDE for critical reading of the manuscript. A. BENSARD assisted us in preparing the manuscript and B. AGOUTIN and T. HEYMANN assisted us in photography; we appreciate this help. This work was supported by Institut Curie and Centre National de la Recherche Scientifique. One of us (E.I.) was supported by a grant from the Ministère de la Recherche et de la Technologie followed by a grant from the Fondation pour la Recherche Médicale.

LITERATURE CITED


Communicating editor: A. G. Hinnebusch