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

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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 (ZELEZNYAKOVA *et al.* 1975; SUSLOVA, FEDOROVA and ZELEZNYAKOVA 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 KOVALTZOVA 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 recom-

ination 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, LICHTEN 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, LICHTEN 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-

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aptonemal 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 *rad50* 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*, *SPO13*, *RAD50* and *RAD52* genes were constructed by the one-step gene disruption method of ROTHSTEIN (1983). To disrupt *XRS2*, plasmid pE140 containing a part of the *XRS2* coding region substituted with a 2.0-kb *HpaI-HpaI* *LEU2* fragment was used (E. IVANOV, unpublished results). pE140 was digested with *Bam*HI and *Hind*III and used to transform recipient strains to *Leu*⁺. To disrupt *SPO13*, plasmid pNKY58 containing a part of the *SPO13* coding region interrupted with a 3.8-kb *Bgl*II-*Bam*HI *hisG-URA3-hisG* fragment was used (ALANI, CAO and KLECKNER 1987). pNKY58 was digested with *Bam*HI and used to transform recipient strains to *Ura*⁺. For disruption of *RAD50*, pNKY83 carrying a complete deletion of *RAD50* substituted with a 3.8-kb *Bgl*II-*Bam*HI *hisG-URA3-hisG* fragment was digested with *Bgl*II and *Eco*RI and used to transform recipient strains to *Ura*⁺ (ALANI, SUBBIAH and KLECKNER 1989). pNKY58 and pNKY83 were constructed in N. KLECKNER's laboratory and were kindly provided for us by Y. KASSIR and W. RAYMOND, respectively. To disrupt *RAD52*, two plasmids pJH181 and pJH183, containing the *RAD52* coding region interrupted with *LEU2* and *URA3*, respectively, were used. Both plasmids were digested with *Bam*HI and used to transform recipient strains to *Leu*⁺ and *Ura*⁺, respectively. pJH181 and pJH183 were a kind gift of D. SCHILD.

Media and growth conditions: Rich medium (YEPD),

synthetic complete medium (SC) with bases and amino acids omitted as specified were as described (SHERMAN, FINK and HICKS 1986). Presporulation medium (YPA) was YEPD with 2% potassium acetate substituted for glucose (MALONE and ESPOSITO 1981). Sporulation medium contained 2% potassium acetate and was supplemented with appropriate nutritional requirements. Inheritance of the *Rad*⁻ phenotype was followed by the inability of cells to grow on YEPD plates supplemented with 0.015% methyl methanesulfonate (MMS) purchased from Sigma. Genetic manipulations were performed according to published procedures (SHERMAN, FINK and HICKS 1986).

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 $1-2 \times 10^7$ cell/ml and incubated for 3-4 days with vigorous shaking. Appropriate dilutions were then plated onto SC-HIS and YEPD plates to measure meiotic levels of recombination.

To assay meiosis-specific double-strand breaks at the *ARG4*, a modification of this protocol was used. A single colony of a SK1 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 $1-2 \times 10^7$ 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°.

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):

$$\text{Rate} = \frac{0.4343 \text{ of median frequency}}{\log(\text{final cell}) - \log(\text{initial cell})}$$

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/m²/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*^R 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*^R 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*^R papillae appeared

TABLE 1
Yeast strains

Strain ^a	Genotype ^b
EI302	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>XRS2</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>XRS2</u>
EI304	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>xrs2-1</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>XRS2</u>
EI303	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>xrs2-1</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>xrs2-1</u>
EI309	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>XRS2</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>XRS2</u>
EI310	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>xrs2-1</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>XRS2</u>
EI311	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>xrs2-1</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>xrs2-1</u>
EI317	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>XRS2</u> <u>rad52::LEU2</u> <u>α</u> <u>his702 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>XRS2</u> <u>RAD52</u>
EI312	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>XRS2</u> <u>rad52::LEU2</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>XRS2</u> <u>rad52::LEU2</u>
EI315	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>xrs2-1</u> <u>rad52::LEU2</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>XRS2</u> <u>rad52::LEU2</u>
EI318	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>xrs2-1</u> <u>rad52::LEU2</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>xrs2-1</u> <u>RAD52</u>
EI314	<u>a</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>LYS1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>xrs2-1</u> <u>rad52::LEU2</u> <u>α</u> <u>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52</u> <u>spo13::URA3</u> <u>xrs2-1</u> <u>rad52::LEU2</u>
EI306	<u>a</u> <u>lys2-1 leu1-12 trp5-2</u> <u>MET4 ura3-1</u> <u>spo13-1</u> <u>XRS2</u> <u>α</u> <u>lys2-1 LEU1 TRP5 met4-1 ura3-1</u> <u>spo13-1</u> <u>XRS2^c</u>
EI305	<u>a</u> <u>lys2-1 leu1-12 trp5-2</u> <u>MET4 ura3-1</u> <u>spo13-1</u> <u>xrs2-1</u> <u>α</u> <u>lys2-1 LEU1 TRP5 met4-1 ura3-1</u> <u>spo13-1</u> <u>XRS2</u>
EI307	<u>a</u> <u>lys2-1 leu1-12 trp5-2</u> <u>MET4 ura3-1</u> <u>spo13-1</u> <u>xrs2-1</u> <u>α</u> <u>lys2-1 LEU1 TRP5 met4-1 ura3-1</u> <u>spo13-1</u> <u>xrs2-1</u>
EI413	<u>a</u> <u>ho::LYS2 leu2-3,112 lys2</u> <u>ura3-52</u> <u>XRS2</u> <u>α</u> <u>ho::LYS2 leu2-3,112 lys2</u> <u>ura3-52</u> <u>XRS2</u>
EI414	<u>a</u> <u>ho::LYS2 leu2-3,112 lys2</u> <u>ura3-52</u> <u>xrs2::LEU2</u> <u>α</u> <u>ho::LYS2 leu2-3,112 lys2</u> <u>ura3-52</u> <u>xrs2::LEU2</u>
EI415	<u>a</u> <u>ho::LYS2 leu2-3,112 lys2</u> <u>ura3-52</u> <u>rad50-KI81-URA3</u> <u>XRS2</u> <u>α</u> <u>ho::LYS2 leu2-3,112 lys2</u> <u>ura3-52</u> <u>rad50-KI81-URA3</u> <u>XRS2^d</u>
EI416	<u>a</u> <u>ho::LYS2 leu2-3,112 lys2</u> <u>ura3-52</u> <u>rad50-KI81-URA3</u> <u>xrs2::LEU2</u> <u>α</u> <u>ho::LYS2 leu2-3,112 lys2</u> <u>ura3-52</u> <u>rad50-KI81-URA3</u> <u>xrs2::LEU2</u>
EI287	<u>a</u> <u>leu2-3,112 lys1-1</u> <u>met5</u> <u>TRP1</u> <u>his1 iso1 ura3 can1</u> <u>XRS2</u> <u>α</u> <u>leu2-3,112 lys1-1</u> <u>MET5</u> <u>trp1-289</u> <u>HIS1 ISO1 URA3 CAN1</u> <u>XRS2</u>
EI288	<u>a</u> <u>leu2-3,112 lys1-1</u> <u>met5</u> <u>TRP1</u> <u>his1 iso1 ura3 can1</u> <u>xrs2-1</u> <u>α</u> <u>leu2-3,112 lys1-1</u> <u>MET5</u> <u>trp1-289</u> <u>HIS1 ISO1 URA3 CAN1</u> <u>XRS2</u>
EI291	<u>a</u> <u>leu2-3,112 lys1-1</u> <u>met5</u> <u>TRP1</u> <u>his1 iso1 ura3 can1</u> <u>xrs2-1</u> <u>α</u> <u>leu2-3,112 lys1-1</u> <u>MET5</u> <u>trp1-289</u> <u>HIS1 ISO1 URA3 CAN1</u> <u>xrs2-1</u>
EI299	<u>a</u> <u>ho</u> <u>ADF6 HIS4</u> <u>his7-2 leu2-3,112 lys1-1</u> <u>LYS2</u> <u>trp1-289 ura3-52</u> <u>xrs2-1</u> <u>α</u> <u>HO</u> <u>ade6</u> <u>his4</u> <u>HIS7</u> <u>LEU2</u> <u>lys1-1 lys2-1</u> <u>trp1-289</u> <u>URA3</u> <u>XRS2</u>
EI300	<u>a</u> <u>ho</u> <u>ADF6</u> <u>his7-2 leu2-3,112 lys1-1</u> <u>trp1-289 ura3-52</u> <u>xrs2-1</u> <u>α</u> <u>HO</u> <u>ade6</u> <u>HIS7</u> <u>leu2-3,112 lys1-1</u> <u>trp1-289</u> <u>URA3</u> <u>XRS2</u>
4A-EI283	<u>α</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>trp1-289 ura3-52</u> <u>XRS2</u>
4A-EI283/DR50	<u>α</u> <u>his7-1 leu2-3,112 lys2-1</u> <u>trp1-289 ura3-52</u> <u>XRS2</u> <u>rad50::URA3</u>

(Table 1 continued on page 654)

TABLE 1

Continued

Strain ^a	Genotype ^b
4A-EI283/DR52	α <i>his7-1 leu2-3,112 lys2-1 trp1-289 ura3-52 XRS2 rad52::URA3</i>
4B-EI283	α <i>his7-1 leu2-3,112 lys2-1 trp1-289 ura3-52 xrs2-1</i>
4B-EI283/DR50	α <i>his7-1 leu2-3,112 lys2-1 trp1-289 ura3-52 xrs2-1 rad50::URA3</i>
4B-EI283/DR52	α <i>his7-1 leu2-3,112 lys2-1 trp1-289 ura3-52 xrs2-1 rad52::URA3</i>
EI326	<u>α <i>his7-1 leu2-3,112 lys2-1 LYS1 trp1-289 ura3-52 XRS2 rad52::URA3</i></u> α <i>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52 XRS2 rad52::URA3</i>
EI322	<u>α <i>his7-1 leu2-3,112 lys2-1 LYS1 trp1-289 ura3-52 XRS2 rad50::URA3</i></u> α <i>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52 XRS2 rad50::URA3</i>
EI323	<u>α <i>his7-1 leu2-3,112 lys2-1 LYS1 trp1-289 ura3-52 xrs2-1 rad52::URA3</i></u> α <i>his7-2 leu2-3,112 LYS2 lys1-1 trp1-289 ura3-52 xrs2-1 rad52::URA3</i>
EI254	<u>α <i>HIS7 leu2-3,112 met5 URA3 xrs2-1 CDC40</i></u> α <i>his7-2 LEU2 MET5 ura3-52 XRS2 cdc40-1</i>

Strains EI302, EI309, EI312, EI317, EI322, EI326 are isogenic. Strains EI304, EI310, EI315 are isogenic. Strains EI303, EI311, EI314, EI318, EI323 are isogenic. Strains 4A-EI283/DR50 and 4A-EI283/DR52 are transformants of strain 4A-EI283. Strains 4B-EI283/DR50 and 4B-EI283/DR52 are transformants of strain 4B-EI283. Strains EI413, EI414, EI415, EI416 are isogenic and derive from the SK1 series of strains which undergo a rapid and synchronous meiosis upon transfer to sporulation medium.

^a All strains have been constructed in this study.

^b Unless otherwise indicated, all haploid strains were *ho* and all diploid strains were *ho/ho*.

^c The origin of the *spo13-1* mutation was strain JJ553 (Y. KASSIR).

^d The origin of the *rad50* allele was strain NKY1001 constructed in N. KLECKNER's laboratory and provided for us by B. DE MASSY.

from the colony prints. To ensure independent origin of every Can^R clone, only one papilla was taken from a colony print. The Can^R 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 hsdΔ5 thi Δ(lac-proAB) F'(traD36 proAB⁺ lacI^q 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, ALANI and KLECKNER (1990) with minor modifications proposed to us by B. DE MASSY. Total DNA was digested with *Bgl*II 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 Appligene 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 EI303. 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, includ-

ing EI303, were dissected and no viable spores were obtained. This means that the spore viability of *xrs2-1/xrs2-1* diploid strains 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 can 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 may affect 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 EI309 and EI310 show typical meiotic increases in recombination. In contrast, despite the fact that mitotic recombination was increased in the *xrs2-1 spo13* strain EI311, no induction in recombination above the mitotic level was seen after exposure to sporulation medium. Similarly, meiosis-specific increase in recombination was not observed in a *xrs2* mutant diploid strain bearing an *arg4* heteroallelic combination (E. IVANOV, unpublished results). Therefore, *xrs2* mutations greatly depress intragenic meiotic recombination.

TABLE 2

Sporulation and spore viability in *xrs2* diploid strains

Relevant genotype	Strain	Percent sporulation	Percent spore viability
A) <i>SPO13</i> strains ^a			
<i>XRS2</i> <i>XRS2</i>	EI302	83	87
<i>xrs2-1</i> <i>XRS2</i>	EI304	77	79
<i>xrs2-1</i> <i>xrs2-1</i>	EI303	10	<0.5
B) <i>spo13Δ</i> strains ^b			
<i>spo13Δ XRS2</i> <i>spo13Δ XRS2</i>	EI309	53	62
<i>spo13Δ xrs2-1</i> <i>spo13Δ XRS2</i>	EI310	49	54
<i>spo13Δ xrs2-1</i> <i>spo13Δ xrs2-1</i>	EI311	60	84
<i>spo13Δ XRS2 rad52Δ</i> <i>spo13Δ XRS2 RAD52</i>	EI317	51	ND ^c
<i>spo13Δ XRS2 rad52Δ</i> <i>spo13Δ XRS2 rad52Δ</i>	EI312	0.6	ND
<i>spo13Δ xrs2-1 rad52Δ</i> <i>spo13Δ XRS2 rad52Δ</i>	EI315	1.5	<2.5
<i>spo13Δ xrs2-1 rad52Δ</i> <i>spo13Δ xrs2-1 rad52Δ</i>	EI314	33	57
<i>spo13Δ xrs2-1 rad52Δ</i> <i>spo13Δ xrs2-1 RAD52</i>	EI318	57	85

^a 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 EI302 and EI304 and 15 tetrads for strain EI303 (see also text).

^b Percent sporulation was calculated as for *SPO13* strains. Spore viability was determined by dissecting 20 dyads for strain EI315 and about 100 dyads each for strains EI309, EI310, EI311, EI314 and EI318.

^c 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

TABLE 3

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

Relevant genotype	Strain	His ⁺ /10 ⁶ viable cells ^a	
		Mitotic	Meiotic
<i>spo13Δ XRS2</i> <i>spo13Δ XRS2</i>	EI309	9.9 ± 1.0	1480 ± 141
<i>spo13Δ xrs2-1</i> <i>spo13Δ XRS2</i>	EI310	6.4 ± 1.0	1242 ± 94
<i>spo13Δ xrs2-1</i> <i>spo13Δ xrs2-1</i>	EI311	69 ± 15	68 ± 7
<i>spo13Δ xrs2-1 rad52Δ</i> <i>spo13Δ xrs2-1 rad52Δ</i>	EI314	1.7 ± 0.3	7.3 ± 4.1

^a Six independent cultures were examined for each strain. The means with standard errors are presented.

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 reductional segregation as well as different types of aberrant segregation (for details, see KLAPHOLZ and ESPOSITO 1980). 1⁺:1⁻ 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 4
Effect of *xrs2-1* mutation on meiotic recombination in *spo13-1* dissected dyads

Relevant genotype	Strain	No. of dyads (<i>P</i> : <i>R</i> : <i>A</i>) ^a			
		Chromosome VII		Chromosome XIV	Chromosome III
		<i>leu1</i>	<i>trp5</i>	<i>met4</i>	<i>MAT</i>
<i>spo13-1 XRS2</i> <i>spo13-1 XRS2</i>	E1306	20:67:16	65:34:4	46:48:9	57:38:8
<i>spo13-1 xrs2-1</i> <i>spo13-1 XRS2</i>	E1305	16:68:32	42:68:6	43:65:8	57:33:26
<i>spo13-1 xrs2-1</i> <i>spo13-1 xrs2-1</i>	E1307	119:1:0	120:0:0	119:0:1	116:0:4

Only dyads containing two viable ascospores are included.

^a Dyads were classified into three types (MALONE and ESPOSITO 1981). *P*, dyads in which both spores have the phenotype of an equational division, i.e., 2⁺:0⁻; *R*, dyads in which spores have the phenotype of a single exchange between a gene and its centromere followed by an equational division, i.e., 1⁺:1⁻; *A*, aberrant dyads which do not fit either the *P* or *R* classification.

WADDELL and ESPOSITO 1985; ENGEBRECHT and ROEDER 1989; HOLLINGSWORTH and BYERS 1989; MENEES and ROEDER 1989; MALONE *et al.* 1991; ROCKMILL and ROEDER 1991; BHARGAVA, ENGEBRECHT and ROEDER 1992). This was interpreted to mean that recombination during the single equational division in *spo13* is somewhat harmful and results in meiotic defects due, most probably, to chromosome loss.

In summary, the *XRS2* gene product is required for normal levels of meiotic interhomologue intragenic recombination (most likely gene conversion) as well as for reciprocal recombination.

Triple mutant diploid strain *spo13 xrs2-1 rad52* sporulates and produces viable spores: Greatly reduced sporulation and spore viability due to *rad52* mutations are not alleviated in the presence of a *spo13* mutation (MALONE and ESPOSITO 1981; MALONE 1983) (compare also strains E1312 and E1317 in Table 2B). However, triple mutant diploids *spo13 rad50 rad52* were able to sporulate and to produce viable spores (MALONE 1983). To test whether *xrs2-1* behaves in this respect similar to *rad50* mutations, the triple mutant diploid strain E1314 (*spo13 xrs2 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 *xrs2-1* 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

FIGURE 1.—Meiosis-specific double-strand breaks (DSBs) at the *ARG4* locus in the wild type (EI413) and *xrs2Δ* (EI414) strains. (A) Relevant map of the *ARG4* region on chromosome VIII (adapted from SUN *et al.* 1989). *ARG4* and two adjacent genes, *DED81* and *DED82* are presented along with two *Bgl*II restriction sites and two meiosis-specific DSBs sites. 1.0-kb *EcoRV*-*Bgl*II fragment used as a probe is also shown. (B) Southern analysis of meiotic DSBs in the wild type and *xrs2Δ* strains. DNAs were prepared from cells prior to (0 hr) and 4.5 hr after entry into meiosis, digested with *Bgl*II and hybridized with a radioactive probe. Positions of parental DNA (5.0 kb) and DSB products at site I (3.5 kb) and site II (1.5 kb) are indicated on the right.

(8 hr) as in sporulating cultures of the wild-type strain EI413. That means that the *xrs2* mutation does not affect the time course of meiotic events. We conclude, therefore, that the *xrs2* deletion mutation reduces or prevents the occurrence of meiosis-specific DSBs at the *ARG4* region.

We also looked at meiosis-specific DSBs in strain EI415 homozygous for the nondeletion allele *rad50s* leading to the accumulation of nonprocessed meiotic DSBs (ALANI, PADMORE and KLECKNER 1990; CAO, ALANI and KLECKNER 1990) and in the double mutant diploid EI416 (*rad50s/rad50s xrs2::LEU2/xrs2::LEU2*). DSBs were observed in the former strain and not seen in the latter (data not shown). That is, the *xrs2* deletion mutation is epistatic to *rad50s* with respect to DSBs.

***xrs2-1* increases spontaneous mitotic intragenic recombination and does not prevent its induction**

TABLE 5

Rates of spontaneous mitotic recombination at the *HIS7* gene

Relevant genotype	Strain	Median frequency, per 10 ⁶ viable cells	Rate, per 10 ⁷ cell divisions
<i>XRS2</i>			
<i>XRS2</i>	EI302	5.9	4.0
<i>xrs2-1</i>			
<i>XRS2</i>	EI304	8.5	5.9
<i>xrs2-1</i>			
<i>xrs2-1</i>	EI303	64.3	45.0

by irradiation: We observed that the *xrs2-1* mutation increased 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 for gamma rays (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.

FIGURE 2.—Cell inactivation (open symbols) and induction of intragenic recombination at the *HIS7* locus (closed symbols) by gamma rays in diploid strains. □, ■, strain EI302 (*XRS2/XRS2*); ○, ●, strain EI304 (*xrs2-1/XRS2*); △, ▲, strain EI303 (*xrs2-1/xrs2-1*). Four independent experiments have been performed; a typical one is presented.

FIGURE 3.—Cell inactivation (open symbols) and induction of intragenic recombination at the *HIS7* locus (closed symbols) by UV light in diploid strains. □, ■, strain EI302 (*XRS2/XRS2*); ○, ●, strain EI304 (*xrs2-1/XRS2*); △, ▲, strain EI303 (*xrs2-1/xrs2-1*). Four independent experiments have been performed; a typical one is presented.

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 ($\chi^2 = 0.78$; $0.25 < P < 0.5$). 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 homozygous 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 EI287 is equal to $(50 \times 10^{-5}) \times 0.08 = 4.0 \times 10^{-5}$, whereas the same values for strain EI291 equals $(3.5 \times 10^{-5}) \times 0.23 = 8.1 \times 10^{-6}$ and for strain EI288, $(2.2 \times 10^{-5}) \times 0.20 = 4.4 \times 10^{-6}$. 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 (EI299 and EI300) 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*, *MAT α* , and N phenotypes (Table 7B). This clearly demonstrates that *xrs2-1* does not prevent

TABLE 6
Effect of the *xrs2-1* mutation on spontaneous mitotic recombination in chromosome V

Relevant genotype	Strain	Can ^R (event per 10 ⁵ cell division)	Phenotypic analysis of Can ^R segregants			
			No. of Can ^R analyzed	Ura ⁺ Iso ⁺	Ura ⁻ Iso ⁺	Ura ⁻ Iso ⁻
<i>XRS2</i> <i>XRS2</i>	EI291	3.5	453	348 (77%)	105 (23%)	0
<i>xrs2-1</i> <i>XRS2</i>	EI288	2.2	464	372 (80%)	92 (20%)	0
<i>xrs2-1</i> <i>xrs2-1</i>	EI287	50.0	466	425 (91%)	37 (8%)	4 (1%) ^a

^a All Iso⁻ clones were also His⁻.

TABLE 7
Mating-type segregation in *MATa/MATα HO/ho XRS2/xrs2-1* diploids

A. Mating phenotypes of tetrads ^a				
Diploid	Tetrads analyzed	2a:2N ^b	2α:2N	1a:1α:2N
EI299	20	5	4	11
EI300	19	4	4	11
Total	39	9	8	22

B. Mating phenotypes of spores						
Spore genotype	Whole tetrads ^c			Total viable spores ^d		
	a	α	N	a	α	N
<i>XRS2</i>	18	22	38	19	25	49
<i>xrs2-1</i>	22	16	40	28	23	46

^a Data obtained from asci with four viable spores.

^b N, nonmating segregants.

^c Data from the same 39 tetrads described in Part A.

^d Data from all viable spores from 52 asci of EI299 and EI300, including those from whole tetrads.

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 homo-

FIGURE 4.—Cell inactivation by gamma rays in haploid strains. Symbols: ●, strain 4A-EI283 (*XRS2*), △, strain 4B-EI283 (*xrs2-1*); □, strain 4A-EI283/DR50 (*rad50::URA3*); ■, strain 4B-EI283/DR50 (*xrs2-1 rad50::URA3*); ◆, strain 4A-EI283/DR52 (*rad52::URA3*); ▲, strain 4B-EI283/DR52 (*xrs2-1 rad52::URA3*). Averaged data from two experiments are presented.

zygous 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 (SAEKI, 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

FIGURE 5.—Cell inactivation by gamma rays in diploid strains. Symbols: ●, strain EI302 (*XRS2/XRS2*); △, strain EI303 (*xrs2-1/xrs2-1*); □, strain EI322 (*rad50::URA3/rad50::URA3*); ■, strain EI323 (*xrs2-1/xrs2-1 rad50::URA3/rad50::URA3*); ◆, strain EI326 (*rad52::URA3/rad52::URA3*). Averaged data from two experiments are presented.

only partially affects diploid-specific repair of γ -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* (KOVALTZOVA *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 EI254 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 (KLAPHOLZ 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* (KLAPHOLZ, WADDELL and ESPOSITO 1985), *RED1* (ROCKMILL and ROEDER 1988, 1990), *HOP1* (HOLLINSWORTH and BYERS 1989), *MEI4* (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), *MEK1/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 (HOLLINGSWORTH and BYERS 1989; HOLLINGSWORTH, GOETSCH and BYERS 1990; ROCKMILL and ROEDER 1988, 1990). The ability of the

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 *XRS2*. 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 intrachromatid 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, *XRS2* 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 *XRS2* 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 (ZAKHAROV, KASSINOVA and KOVALTZOVA 1983). In our experiments, when using a pair of *his7* heteroalleles, no effect of the *xrs2-1* 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 1981) reported that UV-induced gene conversion was not reduced by *rad50-1*, whereas KUPIEC and SIMCHEN (1984) observed complete elimi-

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 KOVALTZOVA 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 RUZINSKI 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*.

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