The Role of DNA Repair Genes in Recombination Between Repeated Sequences in Yeast

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ABSTRACT

The presence of repeated sequences in the genome represents a potential source of karyotypic instability. Genetic control of recombination is thus important to preserve the integrity of the genome. To investigate the genetic control of recombination between repeated sequences, we have created a series of isogenic strains in which we could assess the role of genes involved in DNA repair in two types of recombination: direct repeat recombination and ectopic gene conversion. Naturally occurring (Ty elements) and artificially constructed repeats could be compared in the same cell population. We have found that direct repeat recombination and gene conversion have different genetic requirements. The role of the RAD51, RAD52, RAD54, RAD55, and RAD57 genes, which are involved in recombinational repair, was investigated. Based on the phenotypes of single and double mutants, these genes can be divided into three functional subgroups: one composed of RAD52, a second one composed of RAD51 and RAD54, and a third one that includes the RAD55 and RAD57 genes. Among seven genes involved in excision repair tested, only RAD1 and RAD10 played a role in the types of recombination studied. We did not detect a differential effect of any rad mutation on Ty elements as compared to artificially constructed repeats.

Recombination between homologous sequences located at nonhomologous locations (ectopic recombination) has been studied in yeast, both in vegetative cells and in meiosis, using artificial repeats (Jinks-Robertson and Petes 1986; Lichten et al. 1987; Lichten and Haber 1989) or naturally occurring repeated sequences (Roeder and Fink 1982; Kupiec and Petes 1988a,b; Louis and Haber 1990; Melamed et al. 1992; Parket and Kupiec 1992; Nevo-Caspi and Kupiec 1994). Ectopic reciprocal recombination between repeated sequences located on the same or different chromosomes can create chromosomal aberrations, such as translocations, inversions and deletions. Intrachromosomal recombination can also take place between homologous sequences present in a direct orientation [direct repeat recombination (DRR)] (Jackson and Fink 1981; Klein and Petes 1981; Schiestl et al. 1988).

Ty elements are the main family of natural dispersed repeated sequences in yeast, comprising ~1–2% of the yeast genome; most laboratory strains contain 30–40 copies of this element per haploid genome. Each Ty element is composed of a 5.3-kb central element bracketed by two 0.33-kb long terminal repeats (LTRs). They are members of a widely distributed family of eukaryotic elements similar to retroviruses, the LTR-containing retrotransposons (for a review see Boeke and Sandmeyer 1991). Previous experiments have shown that the spontaneous level of ectopic conversion involving a marked Ty element is low, even though many potential partners, including Ty cDNA, (Melamed et al. 1992) are available for recombination. The vast majority of the conversion events detected is not associated with crossing over (Kupiec and Petes 1988a). DNA-damaging treatments that usually induce mitotic recombination of other sequences do not affect Ty recombination (Parket and Kupiec 1992). Because of their peculiar structure, Ty’s can, in addition to ectopic recombination, engage in DRR between the LTRs (Roeder and Fink 1982; Kupiec and Petes 1988a,b; Parket and Kupiec 1992).

Many radiation-sensitive (rad) mutants have been isolated in yeast. Most of them are defective in DNA repair. The RAD genes can be divided into three epistasis groups: the excision repair group, the recombinational repair group and the error-prone repair group (for recent reviews see Friedberg 1988; Friedberg et al. 1991; Petes et al. 1991; Prakash et al. 1993). In the present study we investigate the role played by genes from the first two groups in recombination between repeated sequences.

The excision repair group of genes is mainly involved in the repair of UV-irradiated DNA. The products of seven genes (RAD1, RAD2, RAD3, RAD4, RAD10, RAD14 and SSL2) are essential for the excision of pyrimidine dimers (Reynolds and Friedberg 1981; Wilcox and Prahask 1981; Prakash et al. 1993). Other genes in this epistasis group, including RAD7, RAD16 and RAD23, are also required for repair of UV-damaged DNA, but
mutations in these genes do not completely abolish dimer excision (Miller et al. 1982).

Genes from the recombinational repair group are mainly involved in repair of damage caused by ionizing radiation. The RAD51, RAD52, RAD54, RAD55 and RAD57 genes belong to this group and display similar phenotypes with respect to damage sensitivity (Saeki et al. 1980, Game 1983, 1993). Among these genes, RAD52 has been extensively studied. rad52 mutants are unable to repair double-strand breaks (DSBs) (Resnick and Martin 1976) and are thus impaired in HO-induced mating type conversion (Malone and Esposito 1980). They are defective in meiotic recombination (Game et al. 1980; Prakash et al. 1981; Zamb and Petes 1981; Ozenberg and Roeder 1991) and are only slightly defective in DRR (Shinohara 1992) and are partially defective in meiotic recombination (reviewed in Malone and Esposito 1980). Rad51 protein physically interacts with the RAD52 gene product (Milne and Weaver 1993). rad51 mutants are defective in mitotic heterallelic recombination in diploids (Sung 1994). The Rad51 protein shares homology with the bacterial RecA protein (Aboussekra et al. 1992; Basile et al. 1992; Shinohara et al. 1992). Like RecA, Rad51 can form a filament on DNA (Ogawa et al. 1993) and carry out a strand-transfer reaction in vitro (Sung 1994). The Rad51 gene product interacts with the RAD52 gene product (Milne and Weaver 1993). Rad51 mutants are defective in mitotic heterallelic recombination in diploids (Saeki et al. 1980, Aboussekra et al. 1992) and are partially defective in meiotic recombination (Borts et al. 1986; Shinohara et al. 1992) but proficient in DRR (Shinohara et al. 1992; McDonald and Rothstein 1994).

The RAD53 gene product shares homology with bacterial RecA proteins (Aboussekra et al. 1992; Basile et al. 1992; Shinohara et al. 1992). Like RecA, Rad53 can form a filament on DNA (Ogawa et al. 1993) and carry out a strand-transfer reaction in vitro (Sung 1994). The Rad53 protein physically interacts with the RAD52 gene product (Milne and Weaver 1993). Rad51 mutants are defective in mitotic heterallelic recombination in diploids (Saeki et al. 1980, Aboussekra et al. 1992) and are partially defective in meiotic recombination (Borts et al. 1986; Shinohara et al. 1992) but proficient in DRR (Shinohara et al. 1992; McDonald and Rothstein 1994).

The RAD55 and RAD57 gene products also share homology with RAD51 and the bacterial RecA protein (Kans and Mortimer 1991; Lovett 1994). The reported phenotypes of rad55 and rad57 mutants with respect to mitotic and meiotic recombination are similar to the rad52 phenotype: they seem to be defective in both processes (Saeki et al. 1980; Lovett and Mortimer 1987; Petes et al. 1991). The RAD54 gene codes for a protein with weak homology to helicases (Emery et al. 1991). rad54 mutants show reduced mitotic heterallelic recombination in diploids (Saeki et al. 1980) but appear to be proficient in meiotic recombination (Game 1983).

Studies have been carried out in many laboratories to elucidate the genetic control of DRR between artificial repeats (Jackson and Fink 1981; Aguilera and Klein 1988, 1989; Klein 1988; Schiestl and Prakash 1988, 1990). These studies have shown that some genes of the excision repair group, such as RAD1 and RAD10, are needed for direct repeat interactions, whereas the RAD52 gene, which belongs to the recombinational repair group, seems to play a small role in this type of recombination. Ectopic conversion between artificial repeats, on the other hand, seems to be dependent on the same set of genes that participates in allelic recombination (Steele et al. 1991). These studies were carried out using different strains and recombination systems. This has made a systematic comparison between the effect of different rad mutations on the different types of recombination difficult. In addition, little is known about the genetic control of recombination between naturally occurring repeats, such as the Ty elements. Because of their distribution and the fact that they are flanked by direct repeats, Ty's can engage in two different types of homologous recombination: the LTRs can interact by direct-repeat recombination and the whole Ty can recombine ectopically with other Ty's in the genome.

In the present study we investigate the role of the RAD genes in different types of mitotic recombination. We have constructed strains in which we can measure Ty and non-Ty DRR, interchromosomal ectopic recombination of an artificial repeat and ectopic recombination of Ty elements. All of these events are scored in the same cell population. We have systematically analyzed the role played by DNA repair genes in the different types of recombination by creating an isogenic series of strains, each defective for a different RAD gene or pair of genes.

MATERIALS AND METHODS

General methods: Standard molecular biology procedures for cloning, restriction analysis, Southern blot analysis, etc. were performed as in Sambrook et al. (1989). Yeast molecular biology procedures were done as previously described (Parker and Kupiec 1992). Synthetic drop-out media (SD) and rich media (YPD) were prepared as described in Sherman et al. (1986). Dropout media lacking one nutrient are designated SD--nutrient (e.g., SD--Ad is SD without adenine). CAN medium is SD--Arg plus 40 μg of canavanine sulfate. CAN plates contained 12 mg/l adenine to allow proper color development of Ade− colonies. 5-fluoro-orotic acid (FOA) medium is SD-complete with 50 μg of uracil and 0.85 g of 5-FOA per liter (Boeke et al. 1984). Strains: All the yeast strains used in the present study are isogenic derivatives of strain MK131. Strain MK131 is a Ura derivative of strain AP5 (Parker and Kupiec 1992) obtained by selection on 5-FOA medium (Boeke et al. 1984). Its genotype is MATa ade2–1 ochre cam1–100 ochre ura3–52 leu2–3,112 trp1Δ lys2::TylSup HIS3::lys2::ura3–x. All the other strains were created by transformation of MK131 or its derivatives as described below.

Strain MK166 (Figure 1A) was constructed by transforming MK131 with plasmid pAP9 to Trp+ prototrophy. This plasmid is based on the integrative vector pRS304 (Sikorsky and Hieter 1989) and carries a 850-bp PvuII-Sacl fragment internal to the HIS5 gene at the Smal cloning site. Upon digestion of pAP9 with BgII, the plasmid integrates at the HIS5 locus to create two nonfunctional copies of the HIS5 gene (His− Trp+ cells). Recombination between the 300-bp direct repeats can regenerate the HIS5 gene, giving a His+ Trp− cell (for a similar system see Schiestl et al. 1988).

Strain RM6 was created in two steps. First, a red (Ade′) CanR derivative of MK131 in which the TylSup was replaced
by a solo LTR was transformed with plasmid pAP7. This plasmid, a derivative of pM77 (PARKET and KUPIEC 1992), carries a hys2::Ty1Sup allele in which the 5' LTR of the Ty has been deleted (up to the HpaI site at position 815 of the Ty) (BOEKE et al. 1988) on a URA3-containing integrative vector. Ura+ Ade+ transformants carrying an integrated pAP7 plasmid were plated on 5-FOA plates (BOEKE et al. 1984) to select for Ura+ Ade+ Can+ colonies in which excision of the plasmid has left deleted a derivative of pM77 (PARKET and KUPIEC 1992), carries a lys2::TylSup allele in which the 5' LTR of the Ty has been replaced with the LEU2 marker (D. SCHILD, personal communication). Plasmid pR14.4 carries a deletion of the SUP4 gene (SCHIESTL and PRAKAS 1983). The following plasmids were used.

Plasmids: pM43, pM32 and pM21, carrying the LYS2, LEU2 and URA3 loci, respectively (KUPIEC and PETES 1988), and pAP1, carrying the SalI fragment containing the SUP4 locus (PARKET and KUPIEC 1992), were used as probes for Southern blot analysis of recombinant colonies.

To create the rad strains, plasmids carrying marked disruptions or deletions of different repair genes were used (ROTHSTEIN 1983). The following plasmids were used.

In plasmids pRR46 and pDG18 the sequences —212 to +3856 of the RAD1 gene (SCHIESTL and PRAKAS 1988) were replaced, respectively, by the LEU2 or URA3 genes (PAETKAU et al. 1994). Plasmid pKM55 carries a replacement of an internal BglII fragment of the RAD2 gene by the URA3 gene (MADURA and PRAKAS 1986). In plasmid pDG39 the KpnI-Bu36I fragment of the RAD4 gene (COUTO and FRIEDBERG 1989) was replaced by the Gene Blaster construct, which contains a URA3 marker (ALANI et al. 1987). Plasmid pDG78 is a replacement of the HindIII-KpnI internal fragment of RAD7 by the URA3 gene (PEROZZI and PRAKAS 1986). Plasmid pDG271 carries a BalI-XbaI deletion of most of the RAD10 gene (PRAKAS et al. 1985) replaced by a URA3 fragment (D. GIEZT, personal communication). Plasmid plR14.4 carries a deletion of +40 to +581 of the RAD14 gene (BANKMANN et al. 1992) replaced by the Gene Blaster construct. pDG28 is a replacement of the BglII fragment of RAD23 (positions +47 to +1110) (WATKINS et al. 1993) by the Gene Blaster construct.

pLS100 is a URA3 insertion into the unique EcoRI of RAD31 (BASILE et al. 1992). pH181 and pH183 are insertions of the LEU2 and URA3 genes, respectively, into the unique BglII site in the RAD52 gene (SCHILD et al. 1985). In pLS101 a BamHI fragment of the RAD54 gene (EMERY et al. 1991) was replaced by a URA3 marker. pST11 is a disruption of RAD55 by insertion of the LEU2 gene into the unique SalI site (LOVETT and MORTIMER 1987). In pSM51 a PvuII-SalI fragment of RAD57 was replaced with the LEU2 marker (D. SCHILD, personal communication).

Strain construction: Transformation was performed by the lithium acetate method (SCHIESTL and GIEZT 1989) after digestion of the plasmids with the proper restriction enzymes. After transformation, a phenotypic screen was performed, when possible, by testing resistance to UV-radiation or resistance to methyl methanesulfonate (MMS) [on YEPD, plates containing 0.01% MMS (Aldrich)]. At least six different candidates were subjected to Southern blot analysis to confirm the proper configuration of the construct. To construct double mutants, when possible, each single mutant strain was transformed with a plasmid that disrupts the other gene, thus creating the same double mutant in two alternative ways.

Recombination and mutation measurements: Recombination, as well as mutations in the CAN1 gene, were measured by fluctuation test, as described (PARKET and KUPIEC 1992), for two or three independent transformants of each strain; each experiment was repeated at least twice.

Briefly, 12–36 similarly sized colonies grown 2–3 days at 30°C on YEPD plates were taken to sterile water or transferred to 5 ml of liquid YEPD and further incubated overnight; after appropriate dilution the cells were plated on YEPD (viable count), SD–Lys, to measure ectopic recombination of the hys2 heteroalleles, and CAN plates, to measure Ty recombination (red colonies) as well as mutations in the CAN1 gene (white colonies). In the case of MK166 and derivatives, colonies were also plated on SD–His plates. Colonies were counted after 3 days. Recombination and mutation rates were calculated by the method of the median (LEA and COULSON 1948). The SDs in each fluctuation test, as well as between experiments, were lower than 25% for each strain.

PCR: All the PCR reactions were carried out in a Minicycler apparatus (MJ Research) starting with a small lump of cells picked with a toothpick from a fresh colony. The cells were transferred to a tube carrying the reaction mix consisting of 50 mM dNTPs, 200 ng of primers and 1X buffer, as recommended by the manufacturer, in a total volume of 50 µl. Following cell lysis (3 min incubation at 97°C), 0.5 units of Taq DNA polymerase (Appligene) were added, and the cells were subjected to 30 cycles of 60 sec at 94°C, 60 sec at 54°C and 90 sec at 72°C. The products were run in 1.2% agarose gels.

The following primers were used: M01, 5'CCACCGGAATTCTCGTTCG', this sequence overlaps the EcoRI site in the LYS2 gene; M02, 5'ACTAGGAGGTTCCCTGTTCC3', this sequence is near the BglII site of LYS2 and in the opposite orientation. M06, 5'GTGATGACAAAAACCTCCTTCCG3', this sequence is internal to Ty1 at positions 5502–5520 (BOEKE et al. 1988). M01 and M02 are homologous to the LYS2 gene, flanking the point of insertion of the Ty1Sup. They produce a 856-bp fragment, derived from the LYS2 insert on chromosome XV, that serves as an internal control and a 989-bp fragment when a solo LTR is left after recombination. No PCR product is seen when a whole Ty is present (a 7kb fragment would have been expected). M01 and M06 give a 876-bp band diagnostic of a Ty element. All the colonies that proved positive for the 876-bp band were subjected to Southern analysis (using a LYS2 probe) to distinguish between those that still have the Ty1Sup and those in which the marked Ty has been replaced by an unmarked one.

RESULTS

Strain MK131 carries a Ty1 element marked both physically and genetically by the insertion of SUP4, a suppressor tRNA (Ty1Sup). This Ty was inserted at the LYS2 locus (Figure 1A). The strain carries two ochre-suppressible mutations, one at the ADE2 locus (ade2–1) and one at the CAN1 gene (can1–100); the presence of the Ty1Sup renders the cells Ade− Can−. Gene conversion events between Ty1Sup and other Ty’s in the genome, or interactions between the LTRs of the Ty that delete the SUP4 information, yield CanA Ade− cells, which can be selected on CAN plates. These colonies are red, due to the accumulation of a red pigment in the Ade− cells. White CanA colonies can also arise as a consequence of secondary mutations in the can1–100 allele. In addition, MK131 also carries two nonfunctional copies of the LYS2 gene: one at chromosome II (lys2−:Ty1Sup) and another on chromosome XV, close to the HIS3 locus (HIS3:lys2−:ura3-x). Ectopic recombination between these two copies produces Lys+ cells that can be selected on SD–Lys plates (Figure 1A). The majority of these events are conversions that erase the smaller ura3− insertion (PARKET and KUPIEC 1992). Thus, MK131 allows us to measure, in the same cell population, Ty recombination [both ectopic conversion
by other Ty's and recombination between the terminal direct repeats (red Can^R colonies), ectopic conversion between non-Ty sequences (Lys^+ colonies) and mutation (white Can^R colonies) (PARKET and KUPIEC 1992). Strain MK166 carries, in addition, a duplication of part of the His^4 gene. This construct resembles a Ty element in size and proportions (a 5-kb fragment flanked by two 300-bp direct repeats) and allows us to measure non-Ty DRR (Figure 1A). Starting with these strains, we created an isogenic series of strains, each defective in the function of one or two RAD genes.

Recombinational repair genes: The RAD51, RAD54, RAD55 or RAD57 genes are involved in the repair of DNA damage through recombination. In strains in which these genes have been disrupted, however, the rate of appearance of red Can^R colonies (Ty recombination) was increased by a factor of 15- to 20-fold compared to that of the RAD control. PCR or Southern blot analysis of independently obtained red Can^R colonies from the RAD strain showed that ~60% of the red Can^R colonies are the result of an event that deletes most of the Ty1Sup leaving a solo LTR, whereas in one-third of the colonies the Ty1Sup has been replaced by an unmarked Ty. The colonies that carry a now single LTR could be produced by several mechanisms, which we collectively refer to as DRR. Among them are the following: 1) intrachromatidal recombination (pop-out), 2) unequal crossing over between chromatids in G2, 3) conversion of the Ty1Sup by a solo LTR, 4) replication slippage, 5) a double strand break (DSB) followed by a one-end-invasion crossover (BELMAAA and CHARTLAND 1994), or 6) single-strand annealing (a DSB followed by single-stranded degradation in both directions and annealing of the complementary ssDNA at the homologous repeats) (LIN et al. 1990; MARYON and CARROLL 1991; FISHEMAN-LOBELL et al. 1992). The second type of colonies, in which the Ty1Sup has been replaced by an unmarked Ty, is probably the result of an ectopic
conversion event involving chromosomal Ty's or Ty cDNA (Melamed et al. 1992; Parket and Kupiec 1992). A minority of the red Can^R colonies (3.4% in the RAD strain) are due to mutations in the SUP4 insert or conversion of the gene by the wild-type copy in the genome (Figure 1A).

Analysis of independently derived red Can^R colonies of rad51, rad54, rad55 and rad57 strains shows that colonies carrying a solo LTR account for >95% of the cases, representing a 25-fold increase in the rate of LTR DRR over that of the RAD control. Results obtained with the his4 (non-Ty DRR) recombination system also show a six- to eightfold increase in DRR in these strains (Table 1A).

The rate of ectopic lys2 recombination varies between different mutants in this group (Table 1A). Strains deleted for the RAD55 and RAD57 gene show nearly wild-type levels of recombination; in rad51 and rad54 strains this type of recombination was reduced 30-fold (Table 1A).

The vast majority of red Can^R colonies observed in a rad52 strain contained unchanged Ty1Sup elements and are thus due to putative mutations in the SUP4 insert and not to recombination events. The level of DRR (both LTR and his) was reduced tenfold, whereas lys2 recombination was completely abolished (the rate was <10^-9 recombinants/cell division). Ectopic Ty conversion was also reduced in rad52 strains at least 30-fold (<1 X 10^-8). Thus, disruption of RAD52 causes a different phenotype from mutations in other genes of the recombinational repair group. In this respect, it is interesting to note that the RAD51 and RAD52 gene products physically interact (Milne and Weaver 1993). Inactivation of either gene, however, leads to very different phenotypes.

The level of LTR recombination in rad51, rad54, rad55 and rad57 strains is elevated 25-fold over that of the wild-type control. Most of the red Can^R colonies analyzed showed a solo LTR replacing the Ty1Sup. Therefore we do not know whether the level of Ty ectopic conversion is reduced in these strains or remains unchanged. In strain RM6 one of the LTRs of the Ty1Sup was deleted, thus preventing LTR DRR; red Can^R colonies can be produced only by conversion or mutation (Figure 1B). Analysis of red Can^R colonies from rad51 and rad54 derivatives showed that conversions of the Ty1Sup by other Ty elements were reduced 11- to 15-fold. Thus, ectopic Ty conversion and ectopic lys2 conversion are similarly affected in rad51 and rad54 strains. The rad55 and rad57 strains, which are only slightly defective in lys2 ectopic conversion, are also proficient in Ty ectopic conversion. As expected, no ectopic Ty conversion was observed among red Can^R colonies of rad52 strains (Table 1, A and B). We conclude that ectopic conversion of Ty elements and ectopic conversion of lys2 are similarly affected by disruptions of the recombinational repair genes.

Excision repair genes: Recombination and mutation rates measured for strains defective for the RAD1, RAD2, RAD4, RAD7, RAD10, RAD14 and RAD23 genes are given in Table 2. The level of ectopic non-Ty conversion (Lys^+ colonies) was not greatly affected by mutations in any of the excision repair genes, with the possible exception of the rad1 strain, in which a slight increase (X1.7) was seen. The rate of appearance of red Can^R colonies was indistinguishable in RAD, rad1, rad7, rad10, rad14 or rad23 strains and was elevated twofold in rad2 and rad4 strains. The rates of Ty DRR and ectopic Ty conversion were unchanged in strains defective in the RAD2, RAD4, RAD7, RAD10, RAD14
and RAD23 genes. Since the rates and distribution of events remained unchanged in the respective mutant strains, we conclude that these excision repair genes do not play a primary role in Ty or non-Ty (lys2) ectopic recombination.

Even though the rate of appearance of red Can⁺ colonies in radl strains was only slightly elevated (×1.5), the distribution of events in radl cells was different from that seen in the Rad⁺ controls. The majority of red Can⁺ colonies were due to conversion events, and only a third were due to LTR DRR. In agreement with this result, a threefold decrease in non-Ty DRR (His⁺) was also seen (Table 2). Thus RAD1 plays a role in DRR for both Ty and non-Ty direct repeats.

The RAD1 and RAD10 gene products form together a ssDNA endonuclease (Sung et al. 1993; Tomkinson et

### TABLE 1
Relative rates of recombination and mutation of strains defective in recombinational repair (RAD = 1)

<table>
<thead>
<tr>
<th>Strain</th>
<th>His⁺ DRRᵃ</th>
<th>LTR DRRᵇ</th>
<th>Lys⁺ conversionᶜ</th>
<th>Unchanged Ty1Supᵇᵈ</th>
<th>Mutation at canl'</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. MK166 derivatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK166 (RAD)</td>
<td>1 (1.0 × 10⁻⁵²)</td>
<td>1 (8.7 × 10⁻⁷)</td>
<td>1 (2.6 × 10⁻⁷)</td>
<td>1 (0.48 × 10⁻⁷)</td>
<td>1 (2.9 × 10⁻⁷)</td>
</tr>
<tr>
<td>rad51</td>
<td>5.8</td>
<td>26</td>
<td>0.03</td>
<td>17</td>
<td>5.9</td>
</tr>
<tr>
<td>rad52</td>
<td>0.11</td>
<td>0.09</td>
<td>&lt;0.002</td>
<td>54</td>
<td>7.0</td>
</tr>
<tr>
<td>rad54</td>
<td>6.3</td>
<td>27</td>
<td>0.03</td>
<td>27</td>
<td>4.0</td>
</tr>
<tr>
<td>rad55</td>
<td>7.6</td>
<td>25</td>
<td>0.46</td>
<td>12</td>
<td>5.7</td>
</tr>
<tr>
<td>rad57</td>
<td>5.8</td>
<td>24</td>
<td>0.75</td>
<td>34</td>
<td>5.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ty conversionᵃ</th>
<th>Unchanged Ty1Supᵇᵈ</th>
<th>Mutation at canl'</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. RM6 derivatives (Ty1Sup with only one LTR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM6 (RAD)</td>
<td>1 (4.9 × 10⁻⁷)</td>
<td>1 (0.28 × 10⁻⁷)</td>
<td>1 (2.4 × 10⁻⁷)</td>
</tr>
<tr>
<td>RMrad51</td>
<td>0.09</td>
<td>16</td>
<td>7.9</td>
</tr>
<tr>
<td>RMrad52</td>
<td>&lt;0.02</td>
<td>18</td>
<td>5.6</td>
</tr>
<tr>
<td>RMrad54</td>
<td>0.07</td>
<td>16</td>
<td>5.9</td>
</tr>
<tr>
<td>RMrad55</td>
<td>0.55</td>
<td>10</td>
<td>6.2</td>
</tr>
<tr>
<td>RMrad57</td>
<td>0.76</td>
<td>6.4</td>
<td>4.6</td>
</tr>
</tbody>
</table>

ᵃ Rate (per cell division) of appearance of His⁺ colonies.
ᵇ Rate of appearance of Lys⁺ colonies.
ᶜ Rate of appearance of white CanR colonies.
ᵈ Rate of mutations at the SUP4 marker or conversion by the sup4 locus.

### TABLE 2
Relative rates of recombination and mutation of MK131 and MK166 derivatives carrying mutations in genes involved in excision repair (RAD = 1)

<table>
<thead>
<tr>
<th>Strain</th>
<th>His⁺ DRRᵃ</th>
<th>LTR DRRᵇ</th>
<th>Ty conversionᶜ</th>
<th>Lys⁺ conversionᶜ</th>
<th>Unchanged Ty1Supᵇᵈ</th>
<th>Mutation at canl'</th>
</tr>
</thead>
<tbody>
<tr>
<td>rad1</td>
<td>0.35</td>
<td>0.69</td>
<td>1.9</td>
<td>1.7</td>
<td>4.8</td>
<td>2.6</td>
</tr>
<tr>
<td>rad10</td>
<td>0.38</td>
<td>0.84</td>
<td>1.0</td>
<td>1.0</td>
<td>8.5</td>
<td>2.0</td>
</tr>
<tr>
<td>rad1 rad10</td>
<td>0.32</td>
<td>0.36</td>
<td>1.8</td>
<td>1.9</td>
<td>13.0</td>
<td>2.6</td>
</tr>
<tr>
<td>rad2</td>
<td>—</td>
<td>1.3</td>
<td>1.2</td>
<td>0.90</td>
<td>7.1</td>
<td>2.2</td>
</tr>
<tr>
<td>rad4</td>
<td>—</td>
<td>1.5</td>
<td>1.1</td>
<td>0.82</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>rad7</td>
<td>—</td>
<td>1.0</td>
<td>0.90</td>
<td>1.0</td>
<td>3.4</td>
<td>1.6</td>
</tr>
<tr>
<td>rad14</td>
<td>—</td>
<td>0.93</td>
<td>0.84</td>
<td>1.0</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>rad23</td>
<td>—</td>
<td>1.1</td>
<td>0.85</td>
<td>0.76</td>
<td>2.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

ᵃ Rate (per cell division) of appearance of His⁺ colonies.
ᵇ Rate of appearance of Lys⁺ colonies.
ᶜ Rate of mutations at the SUP4 marker or conversion by the sup4 locus.
ᵈ Rate of appearance of white CanR colonies.

Calculated by multiplying the rate of red Can⁺ colonies by the proportion of colonies showing the relevant chromosomal configuration. For each strain 19 to 45 independent colonies were analyzed by PCR or Southern blot.
Recombination of Repeated Sequences

**TABLE 3**

Relative rates of recombination and mutation in double mutants derived from MK131 and MK166 (RAD = 1)

<table>
<thead>
<tr>
<th>Strain</th>
<th>His' DRR</th>
<th>LTR DRR</th>
<th>Ty conversion</th>
<th>Lys' conversion</th>
<th>Unchanged TySup⁺</th>
<th>Mutation at can¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>rad1</td>
<td>0.35</td>
<td>0.69</td>
<td>1.9</td>
<td>1.7</td>
<td>4.8</td>
<td>2.6</td>
</tr>
<tr>
<td>rad52</td>
<td>0.11</td>
<td>0.09</td>
<td>&lt;0.02</td>
<td>&lt;0.002</td>
<td>54</td>
<td>7.0</td>
</tr>
<tr>
<td>rad1 rad52</td>
<td>&lt;0.0004</td>
<td>&lt;0.03</td>
<td>&lt;0.06</td>
<td>&lt;0.001</td>
<td>20</td>
<td>1.6</td>
</tr>
<tr>
<td>rad51 rad52</td>
<td>—</td>
<td>&lt;0.09</td>
<td>&lt;0.16</td>
<td>&lt;0.001</td>
<td>47</td>
<td>6.1</td>
</tr>
<tr>
<td>rad52 rad54</td>
<td>—</td>
<td>&lt;0.08</td>
<td>&lt;0.15</td>
<td>&lt;0.002</td>
<td>53</td>
<td>6.4</td>
</tr>
<tr>
<td>rad51</td>
<td>5.8</td>
<td>26</td>
<td>0.09</td>
<td>0.03</td>
<td>17</td>
<td>5.9</td>
</tr>
<tr>
<td>rad54</td>
<td>6.3</td>
<td>27</td>
<td>0.07</td>
<td>0.03</td>
<td>27</td>
<td>4.0</td>
</tr>
<tr>
<td>rad51 rad54</td>
<td>—</td>
<td>23</td>
<td>&lt;2.7</td>
<td>0.04</td>
<td>27</td>
<td>9.3</td>
</tr>
<tr>
<td>rad1 rad51</td>
<td>0.14</td>
<td>0.17</td>
<td>&lt;0.10</td>
<td>0.20</td>
<td>44</td>
<td>9.9</td>
</tr>
<tr>
<td>rad1 rad54</td>
<td>0.21</td>
<td>0.15</td>
<td>&lt;0.14</td>
<td>0.26</td>
<td>40</td>
<td>7.3</td>
</tr>
<tr>
<td>rad35</td>
<td>7.6</td>
<td>25</td>
<td>0.56</td>
<td>0.46</td>
<td>11</td>
<td>5.7</td>
</tr>
<tr>
<td>rad57</td>
<td>5.8</td>
<td>24</td>
<td>1.1</td>
<td>0.75</td>
<td>34</td>
<td>5.3</td>
</tr>
<tr>
<td>rad1 rad55</td>
<td>0.29</td>
<td>0.64</td>
<td>0.28</td>
<td>0.49</td>
<td>26</td>
<td>1.6</td>
</tr>
<tr>
<td>rad1 rad57</td>
<td>0.17</td>
<td>0.22</td>
<td>0.16</td>
<td>0.54</td>
<td>30</td>
<td>2.6</td>
</tr>
<tr>
<td>rad51 rad55</td>
<td>1.7</td>
<td>11</td>
<td>&lt;0.7</td>
<td>0.03</td>
<td>6.6</td>
<td>4.6</td>
</tr>
<tr>
<td>rad51 rad57</td>
<td>1.4</td>
<td>10</td>
<td>&lt;0.6</td>
<td>0.03</td>
<td>6.2</td>
<td>4.0</td>
</tr>
<tr>
<td>rad54 rad55</td>
<td>6.9</td>
<td>26</td>
<td>&lt;1.0</td>
<td>0.05</td>
<td>41</td>
<td>8.6</td>
</tr>
<tr>
<td>rad54 rad57</td>
<td>7.5</td>
<td>24</td>
<td>&lt;1.0</td>
<td>0.04</td>
<td>20</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Rad51 and rad54 mutations are epistatic to rad55 and rad57. Double mutants rad51 rad55, rad51 rad57, rad54 rad55 and rad54 rad57 show levels of Lys⁻ recombinants comparable to those of the individual rad51 or rad54 strains. Similar levels are also seen in the double mutant rad51 rad54 (Table 3). Thus, rad51 and rad54 show identical effects with respect to lys2 conversion. The high level of LTR recombination precluded an accurate estimate of the level of Ty conversion in these strains.

**Analysis of double mutants:** Table 3 shows the results obtained with strains defective in two different RAD genes. The rad52 mutation is epistatic to rad51 or rad54 with respect to lys2 ectopic conversion and to DRR between the LTRs: the RAD52 gene product is absolutely necessary to get LTR recombinants in rad51 and rad54 strains.

The absence of any one of the RAD51, RAD54, RAD55 and RAD57 genes increases the rates of LTR and his4 DRR (Table 1A). Double mutants rad54 rad55 or rad54 rad57 exhibit the same high level of DRR as any of the single mutants. However, double mutants rad51 rad55 and rad51 rad57 show levels of DRR lower than those of the single mutants. This means that the RAD51 gene product is necessary for the high level of DRR seen in rad55 and rad57 strains, whereas the RAD54 gene product is not. Thus, with respect to DRR, RAD51 and RAD54 show distinct interactions with the RAD55 and RAD57 gene products.

For lys2 ectopic recombination, the rad51 and rad54 mutations are epistatic to rad55 and rad57. Double mutants rad51 rad55, rad51 rad57, rad54 rad55 and rad54 rad57 show levels of Lys⁻ recombinants comparable to those of the individual rad51 or rad54 strains. Similar levels are also seen in the double mutant rad51 rad54 (Table 3). Thus, rad51 and rad54 show identical effects with respect to lys2 conversion. The high level of LTR recombination precluded an accurate estimate of the level of Ty conversion in these strains.

**The role of the RAD1 gene in recombination:** rad1 mutants showed a statistically significant decrease in DRR (Table 2), whereas mutations in the RAD51, 54, 55 and 57 genes caused an increase in its rate (Table 1A). Double mutants (rad1 rad51, rad1 rad54, rad1 rad55 and rad1 rad57 strains) do not show the high level of his or LTR DRR seen in the single rad51, rad54, rad55 or rad57 mutants (Table 3). Thus, the RAD1 gene is required for the high level of DRR seen in these strains.

With respect to ectopic conversion, RAD1 interacts with RAD55 and RAD57 in a different way than with...
RAD51 and RAD54. Double mutants rad1 rad53 or rad1 rad57, like the single rad55 and rad57 strains, showed almost wild-type levels of conversion. Thus, most lys2 ectopic recombination can take place in the absence of the RAD1, RAD55 or RAD57 gene products. The double mutants still carry out ectopic Ty conversion at levels comparable to those seen in the rad53 and rad57 single mutants (Table 3).

In contrast, the absence of the RAD1 gene increased the level of lys2 recombination in rad51 and rad54 strains by one order of magnitude, implying that in the absence of Rad51 and Rad55 the RAD1 gene product impairs the production of recombinants; either cells that attempt to recombine die, or the DNA is repaired in a nonrecombinogenic way. The high level of LTR DRR precluded an accurate measurement of Ty conversion in these strains.

The rad52 mutation acts synergistically with the rad1 deletion; no His+ colonies were seen in double mutants rad1 rad52, suggesting that these two genes act on alternative pathways of DRR (Table 3). With respect to ectopic conversion, rad52 is epistatic to rad1: no Lys+ recombinants or Ty conversion events were obtained in the rad1 rad52 double mutant.

Role of RAD genes in mutagenesis: Our isogenic series of strains allowed us to measure forward mutation at the CAN1 gene (white CanR colonies). The rate of appearance of white CanR cells was increased about twofold in rad1, rad2, rad4, rad7 and rad10 strains (Table 2). A higher level of mutations in the CAN1 locus (up to sevenfold increase) was seen for the strains defective in recombinational repair (Table 1). All the double mutants of this class analyzed showed similarly elevated levels of mutation, indicating that they all constitute a single epistasis group with respect to mutagenesis. The high level of white CanR colonies in these strains may be due to repair of spontaneous lesions by a repair system that allows synthesis of DNA across spontaneous damage (Kunz et al. 1990). In the absence of the more accurate excision repair or recombinational repair pathways, the mutagenic pathway can repair those lesions creating a higher level of mutations (von Borstel et al. 1971; Hastings et al. 1976; Quah et al. 1980). We note that the rate of mutation at the CAN1 locus in the double mutants rad1 rad52, rad1 rad55 and rad1 rad57 was lower than that seen in single rad52, rad55 or rad57 mutants, implying that the RAD1 gene product may play a role in creating mutations in these strains (Table 3).

The spontaneous rate of appearance of red CanR colonies showing unchanged Ty configuration (which include ectopic conversion by the sup4 wild-type gene and mutation) was $4.8 \times 10^{-4}$ in strains MK131 and MK166. Only a mild (two- to eightfold) increase in this rate was seen among strains deficient in excision repair. In contrast, the rate was elevated 12–50 times in mutants of the recombinational repair group. Since many of these mutants are defective in ectopic conversion, this type of colony is probably due to mutations in the 89-bp long SUP4 insert. The level of white CanR colonies, caused by mutations in the CAN1 gene, was increased in these strains only by four- to sevenfold. A specific increase in mutation associated with an inverted repeat has been previously reported for rad52 mutants (Rattay and Symington 1994). A possible explanation for this high level of mutation is that potential recombination events were initiated in the absence of recombination genes and were rescued by other error-prone repair pathways. It is possible that the presence of repeats, in either orientation, has an influence on either the amount of spontaneous lesions generated or the way these are processed. In this respect it is interesting to note that RM6 and its rad derivatives showed lower levels of mutations in the SUP4 insert than the isogenic strains in which the SUP4 insert is bracketed by LTRs (Table 1).

**DISCUSSION**

We have analyzed the role of several RAD genes on different types of recombination between natural and artificial repeats. Our results can be summarized as follows. 1) Recombination between direct repeats (DRR) and ectopic gene conversion have different genetic requirements. 2) The genes in the recombinational repair group can be divided into three different phenotypic subgroups according to their role in DRR and ectopic conversion and the interactions among them and with the RAD1 gene. 3) Genes in the excision repair group, with the exception of RAD1 and RAD10, do not play a role in the types of recombination monitored. 4) We did not detect a differential effect of any rad mutation on Ty elements, as compared to artificially constructed repeats.

The recombinational repair genes analyzed fall into three groups: The repair phenotype of the mutants of the recombinational repair group is quite similar; however, they vary in their ability to carry out different types of recombination. We have analyzed the role of the RAD51, RAD52, RAD54, RAD55 and RAD57 genes in ectopic Ty and lys2 recombinational DRR between his4 repeats or LTRs. Based on our results we can divide them into three phenotypic subgroups.

The RAD52 gene plays a clear central role in most types of recombination analyzed; in its absence ectopic lys2 recombinational repair is reduced by at least 500-fold. We have not recovered a single Lys+ colony out of a strain carrying a mutation at the RAD52 gene. Similarly, the level of ectopic conversion between Ty elements is reduced by at least 30-fold. In contrast, the rad52 mutation decreases DRR by only 10-fold for both the his4 or LTR systems. These results are in accordance with those from other labs (Jackson and Fink 1981; Klein 1988; Schiestl and Prakash 1988; Thomas and Rothstein 1989; McDonald and Rothstein 1994) that show a
lesser effect of the absence of RAD52 on DRR. These results support the hypothesis that the RAD52 gene does not participate in all the pathways involved in DRR. It has been recently proposed (Prado and AguilerA 1995) that DRR occurring by one-ended invasion, but not by SSA, may be dependent on LERA 1995) that DRR occurring by one-ended invasion, DRR. It has been recently proposed (Prado and A圭-

As seen before for other direct repeat systems (Klein 1988; Schiestl and Prakash 1988; Thomas and Rothstein 1989; McDonald and Rothstein 1994), the radl and rad52 mutations show a synergistic interaction with respect to his4 DRR; whereas radl and rad52 strains show 3- and 10-fold reduced levels, respectively, the absence of both gene products reduces the yield of recombinants by three orders of magnitude. LTR recombination seems to behave similarly (Table 3). This implies that these genes act in alternative repair pathways: in the absence of one of them the other can compensate, but lack of both systems drastically reduces the level of recombinants recovered.

The second group is composed of the RAD51 and RAD54 genes. Mutations in these genes cause an increase in the rate of DRR in both the his4 and LTR systems. Similar results have been also reported by McDonald and Rothstein (1994).

The RAD51 and RAD54 genes seem to play a similar role in all types of recombination tested. In the absence of either gene there is a decrease in the rate of ectopic conversion and an increase in the level of DRR. This increase is dependent on RAD52 (see above) and also on RAD1. However, in contrast to radl rad52 double mutants, radl rad51 and radl rad54 strains still show some DRR (Table 3).

Ectopic lys2 and Ty conversion depend on the RAD51 and RAD54 gene products, although some conversion can still take place in their absence. The fact that the rad1 rad51 and rad1 rad54 double mutants show 10-fold higher levels of lys2 ectopic conversion than the rad51 or rad54 single mutants implies that in the single mutants the presence of RAD1 causes a reduction in Lys+ recombinants; potential intermediates in conversion, such as ssDNA, may be eliminated by the Radl endonuclease (Sung et al. 1993; Tomkinson et al. 1993).

Although the RAD51 and RAD54 genes share all the above-mentioned characteristics, the interactions of each one with the third group (RAD55 and RAD57) with respect to DRR are different. Whereas rad54 rad55 or rad54 rad57 strains show the same elevated levels of DRR as the single mutants, rad51 rad55 and rad51 rad57 strains show a lower level of his4 or LTR recombination (the rates are still higher than those of the RAD strain) (Table 3). We conclude that the full increase in DRR can take place in the absence of the RAD54, RAD55 and RAD57 gene products, but in the absence of RAD51, the Rad55 or Rad57 proteins are required and vice versa. These three gene products share homology to the bacterial RecA protein (see Introduction). The formation of a filament on DNA may be a requirement for the high level of DRR.

The third group is composed of the RAD55 and RAD57 genes. The gene products of these genes are not needed for most ectopic lys2 recombination. Ty conversion can also take place in their absence (Table 1B) (Nevo-Caspi and Kupiec 1994). The fact that rad55 and rad57 strains show a similar phenotype with respect to DRR as rad51 and rad54 strains, but a very different one with respect to ectopic recombination between lys2 heteroalleles, indicates that the genetic requirements of DRR and ectopic conversion are different.

rad55 and rad57 mutants are cold sensitive for the repair of ionizing radiation (Lovett and Mortimer 1987) and for recombination between inverted repeats (Rattray and Symington 1995). We have also observed a similar trend for DRR in our rad55 and rad57 strains: a high level of DRR is seen at 25° or 30° but not at 37°. In contrast, rad51 and rad54 strains showed elevated levels of DRR at all the temperatures analyzed (data not shown).

Role of excision repair genes in recombination: Among seven genes belonging to the excision repair group, only RAD1 and RAD10 appeared significant for the recombinational events studied here. Deletion of RAD2, RAD4, RAD7, RAD14 or RAD23 had little effect on either the level or distribution of recombination events in lys2 or Ty1Sup.

The RAD1 and RAD10 gene products are involved in recombination between some types of direct repeats (Klein 1988; Schiestl and Prakash 1988, 1990; Thomas and Rothstein 1989). Their gene products form a single-strand endonuclease (Sung et al. 1993; Tomkinson et al. 1993) that cleaves artificial substrates at the junction between duplex and single-stranded DNA containing 3’ tails (Bardwell et al. 1994b). Mutations destroying this activity lower the level of DRR and increase the rate of conversion (Table 2). Interestingly, strains lacking the RAD2 gene product, an endonuclease able to nick similar structures on the 5’ single-stranded end (Habrank et al. 1993; Harrington and Lieber 1994), are proficient for all types of recombination tested. DNA molecules with 3’ single-strand extensions (but not 5’ extensions) are expected as intermediates in single-strand annealing (Lin et al. 1984; Maryon and Carroll 1991; Fishman-LOBell et al. 1992), gap repair (Szostak et al. 1983) or one-ended invasion mechanisms (Belmaaza and Chartrand 1994). The RAD1 and RAD10 gene products are thus probably needed for successful processing of a recombination intermediate to give a single repeat. Experiments carried out with plasmid-borne direct repeats (Fishman-LOBell and HABER 1992) suggest a role for the RAD1 gene in the removal of heterologies at the DNA ends during DRR initiated by a DSB.

The RAD1 and RAD10 gene products, however, are not essential for spontaneous DRR. This implies that
more than one process can act to create the solo LTR or the reconstituted HIS4 gene and the Rad1-Rad10 endonuclease may be needed for only some of these mechanisms.

In the last years some understanding has been achieved on the way several of the excision repair genes act. For example, the Rad2 and Rad4 proteins interact with the yeast transcription factor b (TFIIH), which is essential for proper activity of RNA PolII (BARDWELL et al. 1994a). RAD7 codes for a protein that does not seem to interact with this complex (BARDWELL et al. 1994a) but plays a role in repair of transcriptionally inactive DNA and of the nontranscribed strand of active genes (PAETKAU et al. 1994; VERHAGE et al. 1994). The RAD14 gene codes for a metalloprotein that binds to damaged DNA (GUZDER et al. 1993); RAD23 codes for a protein with a ubiquitin-like domain (WATKINS et al. 1993). Thus, these genes represent diverse aspects of the excision repair process; the fact that none of them has a profound effect in our assays suggests that the nucleotide excision repair system per se is not directly involved in recombination.

Interactions between gene products: Our results show a complex array of interactions between the RAD genes. Although several explanations are possible for the genetic data, the following hypothesis is consistent with our results regarding these interactions.

When an initiating event occurs in a region containing direct repeats, it can be processed in (at least) two ways, one involving directly repeated sequences on the same chromosome, whose final product is a deletion event (DRR) and another that screens the genome for homology and whose final product is a conversion event. These two types of repair may share common steps (FISHMAN-LOBELL et al. 1992; PARKET et al. 1995) but are distinguishable by their different genetic requirements.

At least two different pathways contribute to DRR. The RAD52 and RAD1 genes participate in alternative mechanisms; the action of either of these two genes is essential for DRR, and the double mutant shows at least a 2500-fold reduction in this type of events. The net increase in the rate of DRR observed in rad51, rad54, rad55 and rad57 mutants contrasts with the results seen in rad1 strains, where the rates of Ty recombination were largely unchanged but the distribution of events was altered. It is possible to explain the results obtained in rad1 strains by assuming that DRR and conversion compete for a set number of spontaneous lesions; in the absence of RAD1 more lesions are processed to give conversion events. The results obtained for rad51 and rad54 can be explained in a similar way, since they show a reduction in conversion and a concomitant increase in DRR. Rad55 and rad57, however, show the same increase in DRR without a reduction in ectopic conversion, and rad10 strains show decreased DRR levels without an increase in conversion.

Therefore, the inverse correlation between the rates of DRR and conversion does not always hold.

The net increase in recombinants seen in rad51, rad54, rad55 and rad57 can be explained in two ways. Either the lack of these gene products creates secondary lesions that are repaired by DRR (in other words, there are more initiating events), or the absence of these gene products does not create new lesions. In wild-type strains a vast majority of the initiating lesions are repaired by alternative silent ways, such as sister chromatid repair and are not detected as recombination products. When either RAD51, RAD54, RAD55 or RAD57 are absent, however, all the lesions are repaired by DRR, which acts as a salvage mechanism, and are thus fully detected. By this model, RAD55 and RAD57 should code for proteins involved in silent repair that have no role in ectopic conversion.

Although evidence for preferential repair by sister chromatids in irradiated cells exists (KADYK and HARTWELL 1992 and references therein), we favor the first hypothesis, since the net increase in DRR observed (~25-fold in LTR DRR) would imply that the vast majority (96%) of the spontaneous repair should be silent; sister chromatid repair, however, is not possible in haploid cells during G1, which may comprise a respectable period of the cell cycle. We cannot rule out, however, the possibility that part of the increase observed in rad51, rad54, rad55 and rad57 strains is due to the inactivation of a preexisting silent mechanism rather than to the induction of new recombinational lesions.

By the induction hypothesis then, in the absence of RAD51, RAD54, RAD55 or RAD57, new lesions are created, which are processed by pathways that include the RAD1, RAD10 and RAD52 gene products and give rise to high levels of the deletion product (DRR). In the absence of RAD51, RAD54, RAD55 or RAD57 and of the Rad1 and Rad52 proteins, no new lesions are created, or the lesions created cannot be processed and cause cell death.

Our results also point to a negative effect of the RAD1 gene product on ectopic lys2 conversion in the absence of RAD51 and RAD54. Processing of the new lesions by RAD1 when no nearby homology is present leads to abortive repair and cell death. If a repeat is available, DRR ensues. This may explain why in rad1 rad51 and rad1 rad54 strains DRR levels are lower and conversion levels higher than in rad51 or rad54 single mutants (Table 3).

In recent studies involving inverted repeat recombination (IRR), RATTRAY and SMINGTON (1994, 1995) found an essential role for the RAD52 gene, whereas the RAD51, RAD54, RAD55 and RAD57 genes fall within one category, all having a lower effect, mainly in conversion. The present study further separates these four genes into two functional groups, one comprising the RAD51 and RAD54 genes, and another composed of RAD55 and RAD57. Several interesting similarities and
differences can be found when IRR is compared to DRR and ectopic conversion.

The rad1 mutation by itself showed no effect on IRR but had lower recombination levels in combination with a mutation in the RAD51 gene, implying that in the absence of the RAD51 gene product, the Rad1 protein is involved in IRR (RATTRAY and SYMINGTON 1995) as it is in DRR in our study. Mutations in the RAD55 and RAD57 genes greatly affected the rate of gene conversion between inverted repeats (RATTRAY and SYMINGTON 1995) but did not have an effect on ectopic by2 and Ty conversion (this study). These differences indicate that the genetic requirements for conversion of closely located regions may differ from those involving homology in different chromosomes.

**Genetic control of recombination between naturally occurring repeats**: Our results show that recombination between the Ty’s LTRs follows the same rules as that of an artificially created his4 construct. The particular structure of the Ty elements may be critical in determining their copy number in the genome. Spontaneous LTR recombination could serve in balancing the constant increase in copy number due to transposition. The large numbers of solo LTRs in the genome are probably remnants of this type of cycle: a transposition event to a new genomic location is followed by DRR that leaves a solo LTR (ROEDER and FINK 1982; BOEKE and SANDMEYER 1991).

Ectopic conversion between naturally occurring repeated sequences plays a strong role in their common evolution (EGEL 1981; KUPIEC and PETES 1988; KASS et al. 1995). The genetic control of conversion between Ty elements is remarkably similar to that of the control by2 artificial repeats; thus the differences between the two systems, such as lack of reciprocal recombination (KUPIEC and PETES 1980) and lack of induction by DNA damage (PARKET and KUPIEC 1992) for the Ty system, cannot be attributed to the differential action of any of the genes analyzed in this study. Further investigation is needed to identify the factor(s) responsible for these Ty features.

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Recombination of Repeated Sequences


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