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 PRAKASH 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

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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.* 1980) and several types of mitotic recombination (reviewed in MALONE *et al.* 1988; PETES *et al.* 1991); however, *rad52* mutants are not defective in rDNA recombination (PRAKASH and TAILLON-MILLER 1981; ZAMB and PETES 1981; OZENBERG and ROEDER 1991) and are only slightly defective in DRR (JACKSON and FINK 1981; KLEIN and PETES 1981; KLEIN 1988; SCHIESTL and PRAKASH 1988; THOMAS and ROTHSTEIN 1989; MCDONALD and ROTHSTEIN 1994).

The *RAD51* gene product shares homology with bacterial RecA proteins (ABOUSSEKHRA *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 protein physically interacts with the *RAD52* gene product (MILNE and WEAVER 1993). *rad51* mutants are defective in mitotic heteroallelic recombination in diploids (SAEKI *et al.* 1980, ABOUSSEKHRA *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 *rad51* 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 heteroallelic 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 (PARKET and KUPIEC 1992). Synthetic drop-out media (SD) and rich media (YEPD) were prepared as described in SHERMAN *et al.* (1986). Dropout media lacking one nutrient are designated SD−nutrient (*e.g.,* SD−Ade 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-fluoroorotic acid (FOA) medium is SD-complete with 50 mg 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 (PARKET and KUPIEC 1992) obtained by selection on 5-FOA medium (BOEKE *et al.* 1984). Its genotype is *MATα ade2–1ochre can1–100ochre ura3–52 leu2–3,112 trp1Δ lys2::Ty1Sup 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 300-bp *Pvu*II-*Sca*I fragment internal to the *HIS4* gene at the *Sma*I cloning site. Upon digestion of pAP9 with *Bgl*II, the plasmid integrates at the *HIS4* locus to create two nonfunctional copies of the *HIS4* gene (His$^-$ Trp$^+$ cells). Recombination between the 300-bp direct repeats can regenerate the *HIS4* 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$^-$) Can^R derivative of MK131 in which the Ty1Sup was replaced

by a solo LTR was transformed with plasmid pAP7. This plasmid, a derivative of pM77 (PARKET and KUPIEC 1992), carries a *lys2::Ty1Sup* allele in which the 5′ LTR of the Ty has been deleted (up to the *Hpa*I 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^S colonies in which excision of the plasmid has left a Ty1Sup with a single LTR (Figure 1B). All the configurations were confirmed by Southern blot analysis.

Plasmids: pM43, pM32 and pM21, carrying the *LYS2, LEU2* and *URA3* loci, respectively (KUPIEC and PETES 1988), and pAP1, carrying the *Sal*I 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 PRAKASH 1988) were replaced, respectively, by the *LEU2* or *URA3* genes (PAETKAU *et al.* 1994). Plasmid pKM55 carries a replacement of an internal *Bgl*II fragment of the *RAD2* gene by the *URA3* gene (MADURA and PRAKASH 1986). In plasmid pDG39 the *Kpn*I-*Bsu*36I 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 *Hind*III-*Kpn*I internal fragment of *RAD7* by the *URA3* gene (PEROZZI and PRAKASH 1986). Plasmid pDG271 carries a *Bal*I-*Xba*I deletion of most of the *RAD10* gene (PRAKASH *et al.* 1985) replaced by a *URA3* fragment (D. GIETZ, personal communication). Plasmid pR14.4 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 *Bgl*II fragment of *RAD23* (positions +47 to +1110) (WATKINS *et al.* 1993) by the Gene Blaster construct. pLS100 is a *URA3* insertion into the unique *Eco*RI of *RAD51* (BASILE *et al.* 1992). pJH181 and pJH183 are insertions of the *LEU2* and *URA3* genes, respectively, into the unique *Bgl*II site in the *RAD52* gene (SCHILD *et al.* 1983). In pLS101 a *Bam*HI fragment of the *RAD54* gene (EMERY *et al.* 1991) was replaced by a *URA3* marker. pSTL11 is a disruption of *RAD55* by insertion of the *LEU2* gene into the unique *Sal*I site (LOVETT and MORTIMER 1987). In pSM51 a *Pvu*II-*Sal*I 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 GIETZ 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 disruption. 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° on YEPD plates were taken to sterile water or transfered 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 *lys2* 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 1× buffer, as recommended by the manufacturer, in a total volume of 50 μl. Following cell lysis (3 min incubation at 97°), 0.3 units of *Taq* DNA polymerase (Appligene) were added, and the cells were subjected to 30 cycles of 60 sec at 94°, 60 sec at 54° and 90 sec at 72°. The products were run in 1.2% agarose gels.

The following primers were used: MO1, 5′CCAGCGGAATTCCACTTG3′, this sequence overlaps the *Eco*RI site in the *LYS2* gene; MO2, 5′AACTGAGGGGTCCTTTCC3′, this sequence is near the *Bgl*II site of *LYS2* and in the opposite orientation. MO6, 5′GTGATGACAAAACCTCTTCCG3′, this sequence is internal to Ty1 at positions 5502–5520 (BOEKE *et al.* 1988). MO1 and MO2 are homologous to the *LYS2* gene, flanking the point of insertion of the Ty1Sup. They produce a 656-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 7-kb fragment would have been expected). MO1 and MO6 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^S. 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 Can^R 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 Can^R 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

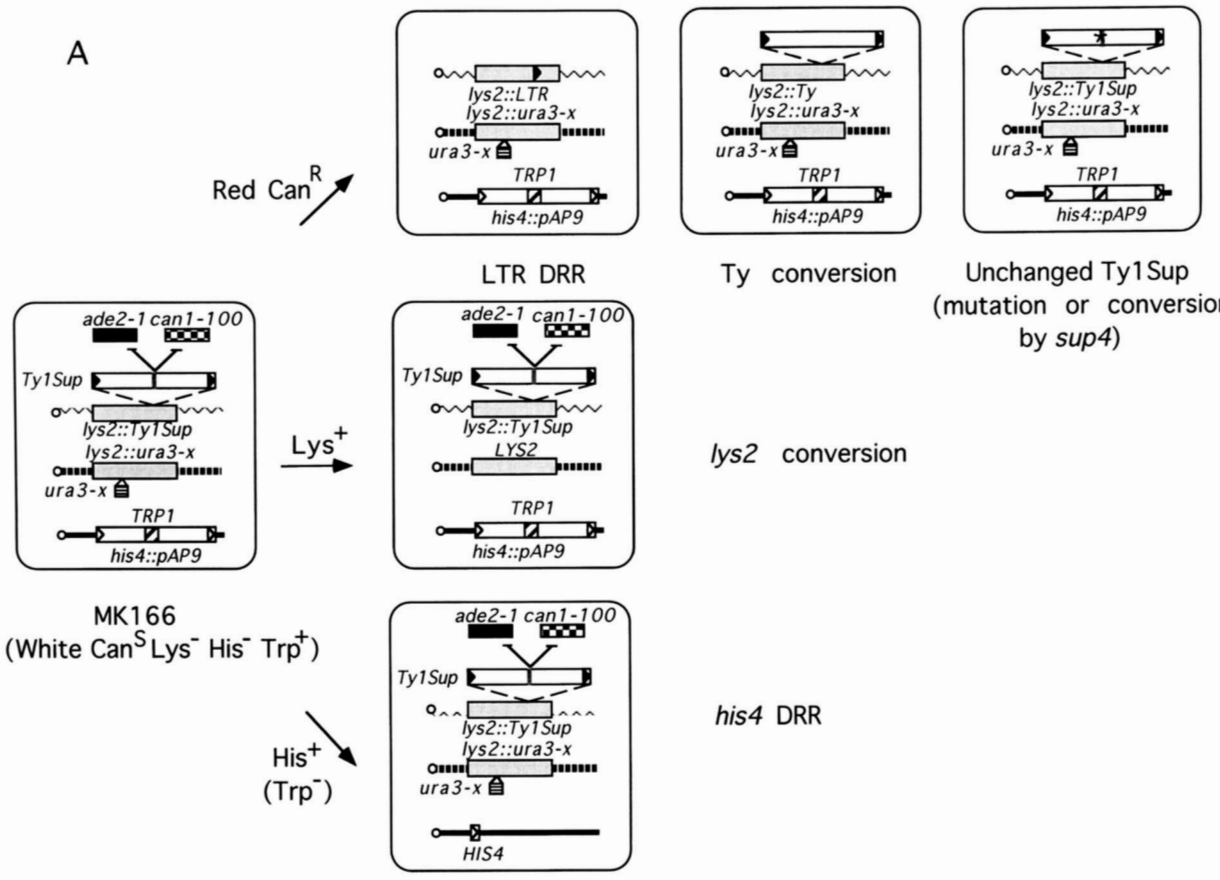

FIGURE 1.—Schematic representation of the genetic systems used in the present study. (A) The strain MK166 carries two copies of the *LYS2* gene (gray rectangles) disrupted by the insertion of either a *Ty1Sup* (chromosome *II*) or a *ura3-x* allele (chromosome *XV*). The presence of the *SUP4* insert in the *Ty1Sup* suppresses the *ade2–1* and *can1–100* alleles, giving a white (Ade$^+$) Can^S phenotype. The two black triangles represent the LTRs of the marked Ty and the *SUP4* insert in the Ty is shown in gray. Red Can^R colonies can be obtained by DRR between the LTRs, ectopic conversion by an unmarked Ty element, conversion of the *SUP4* marker by *sup4* or mutation of *SUP4*. Lys$^+$ colonies are usually created by conversion of the *lys2::ura3-x* to *LYS2* (PARKET and KUPIEC 1992). The integration of the plasmid pAP9 on chromosome *III* creates a 300-bp *his4* duplication (open triangles) separated by vector and *TRP1* sequences. Recombination between the direct repeats recreates a *HIS4* gene; since the *TRP1* information is lost, recombinants are His$^+$ Trp$^-$. (B) In RM6 one of the LTRs has been deleted. Red Can^R colonies can be obtained only by conversion by an unmarked Ty, mutation in the *SUP4* insert, or conversion by *sup4*.

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 *HIS4* 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 (BELMAAZA and CHARTRAND 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; FISHMAN-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

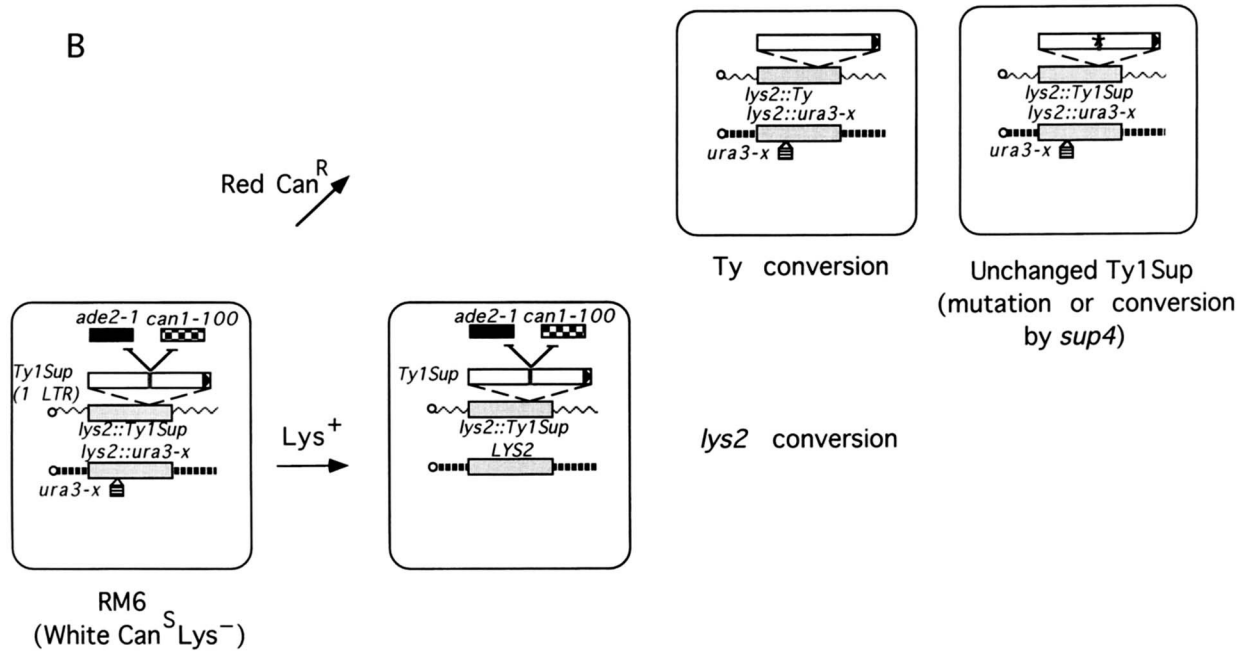

FIGURE 1.—*Continued*

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 ($<1 \times 10^{-8}$). 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 (×1.7) 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*

TABLE 1

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

Strain	His$^+$ DRR[a]	LTR DRR[b]	Lys$^+$ conversion[c]	Unchanged Ty1Sup[b,d]	Mutation at *can1*[e]
			A. MK166 derivatives		
MK166 (*RAD*)	1 (1.0×10^{-5})	1 (8.7×10^{-7})	1 (2.6×10^{-7})	1 (0.48×10^{-7})	1 (2.9×10^{-7})
rad51	5.8	26	0.03	17	5.9
rad52	0.11	0.09	<0.002	54	7.0
rad54	6.3	27	0.03	27	4.0
rad55	7.6	25	0.46	12	5.7
rad57	5.8	24	0.75	34	5.3

Strain	Ty conversion[b]	Unchanged Ty1Sup[b,d]	Mutation at *can1*[e]
	B. RM6 derivatives (Ty1Sup with only one LTR)		
RM6 (*RAD*)	1 (4.9×10^{-7})	1 (0.28×10^{-7})	1 (2.4×10^{-7})
RM*rad51*	0.09	16	7.3
RM*rad52*	<0.02	18	5.6
RM*rad54*	0.07	16	5.9
RM*rad55*	0.55	10	6.2
RM*rad57*	0.76	6.4	4.6

[a] Rate (per cell division) of appearance of His$^+$ colonies.
[b] Calculated by multiplying the rate of red Can^R colonies by the proportion of colonies showing the relevant chromosomal configuration. For each strain 58 to 121 (MK166 derivatives) or 18 to 33 (RM6 derivatives) independent colonies were analyzed by PCR or Southern blot.
[c] Rate of appearance of Lys$^+$ colonies.
[d] Rate of mutations at the *SUP4* marker or conversion by the *sup4* locus.
[e] Rate of appearance of white Can^R colonies.

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^R colonies in *rad1* strains was only slightly elevated (×1.5), the distribution of events in *rad1* cells was different from that seen in the Rad$^+$ controls. The majority of red Can^R 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 2

Relative rates of recombination and mutation of MK131 and MK166 derivaties carrying mutations in genes involved in excision repair (*RAD* = 1)

Strain	His$^+$ DRR[a]	LTR DRR[b]	Ty conversion[b]	Lys$^+$ conversion[c]	Unchanged Ty1Sup[b,d]	Mutation at *can1*[e]
rad1	0.35	0.69	1.9	1.7	4.8	2.6
rad10	0.38	0.84	1.0	1.0	8.5	2.0
rad1 rad10	0.32	0.36	1.8	1.9	13	2.6
rad2	—	1.3	1.2	0.90	7.1	2.2
rad4	—	1.5	1.1	0.82	2.3	2.4
rad7	—	1.0	0.90	1.0	3.4	1.6
rad14	—	0.93	0.84	1.0	1.8	1.0
rad23	—	1.1	0.85	0.76	2.5	1.0

[a] Rate (per cell division) of appearance of His$^+$ colonies.
[b] Calculated by multiplying the rate of red Can^R 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.
[c] Rate of appearance of Lys$^+$ colonies.
[d] Rate of mutations at the *SUP4* marker or conversion by the *sup4* locus.
[e] Rate of appearance of white Can^R colonies.

TABLE 3

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

Strain	His$^+$ DRR[a]	LTR DRR[b]	Ty conversion[b]	Lys$^+$ conversion[c]	Unchanged Ty1Sup[b,d]	Mutation at *can1*[e]
rad1[f]	0.35	0.69	1.9	1.7	4.8	2.6
rad52[f]	0.11	0.09	<0.02	<0.002	54	7.0
rad1 rad52	<0.0004	<0.03	<0.06	<0.001	20	1.6
rad51 rad52	—	<0.09	<0.16	<0.001	47	6.1
rad52 rad54	—	<0.08	<0.15	<0.002	53	6.4
rad51[f]	5.8	26	0.09	0.03	17	5.9
rad54[f]	6.3	27	0.07	0.03	27	4.0
rad51 rad54	—	23	<2.7	0.04	27	9.3
rad1 rad51	0.14	0.17	<0.10	0.20	44	9.9
rad1 rad54	0.21	0.15	<0.14	0.26	40	7.3
rad55[f]	7.6	25	0.56	0.46	11	5.7
rad57[f]	5.8	24	1.1	0.75	34	5.3
rad1 rad55	0.29	0.64	0.29	0.49	26	1.6
rad1 rad57	0.17	0.22	0.16	0.54	30	2.6
rad51 rad55	1.7	11	<0.7	0.03	6.6	4.6
rad51 rad57	1.4	10	<0.6	0.03	6.2	4.0
rad54 rad55	6.9	26	<1.0	0.03	41	8.6
rad54 rad57	7.5	24	<1.0	0.04	20	6.9

[a] Rate (per cell division) of appearance of His$^+$ colonies.
[b] Calculated by multiplying the rate of red Can^R colonies by the proportion of colonies showing the relevant chromosomal configuration. For each strain 30 to 47 independent colonies were analyzed by PCR or Southern blot.
[c] Rate of appearance of Lys$^+$ colonies.
[d] Rate of mutations at the *SUP4* marker or conversion by the *sup4* locus.
[e] Rate of appearance of white Can^R colonies.
[f] Data from Tables 1 and 2, shown for comparison.

al. 1993). Like deletion of the *RAD1* gene, deletion of *RAD10* caused a decrease in *his4* DRR; however, *lys2* and Ty conversion were not increased in the *rad10* strain (Table 2). Whereas the distribution of events among *rad1* red Can^R colonies analyzed was statistically different from that of the Rad$^+$ control, *rad10* results were not different from those of either the Rad$^+$ or *rad1* strains. We interpret these results to imply that apart from their common role as a endonuclease, the individual *RAD1* and *RAD10* proteins may have additional roles, *RAD1* affecting conversion more than *RAD10*. The double mutant *rad1 rad10* showed a phenotype similar to that of the *rad1* strain: a reduced level of DRR and increased Ty and *lys2* conversion (Table 2).

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 rad55* 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 *rad55* 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 Can^R colonies). The rate of appearance of white Can^R 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 Can^R 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 Can^R colonies showing unchanged Ty configuration (which include ectopic conversion by the *sup4* wild-type gene and mutation) was 4.8×10^{-8} 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 Can^R 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 (RATTRAY 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* recombination and 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* recombination 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 *RAD52*.

As seen before for other direct repeat systems (KLEIN 1988; SCHIESTL and PRAKASH 1988; THOMAS and ROTHSTEIN 1989; MCDONALD and ROTHSTEIN 1994), the *rad1* and *rad52* mutations show a synergistic interaction with respect to *his4* DRR; whereas *rad1* 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 *rad1 rad52* double mutants, *rad1 rad51* and *rad1 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 Rad1 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 23° 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 (HABRAKEN *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-LOBEL *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 one 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 SYMINGTON (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 *lys2* 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 as a mechanism to balance 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 *lys2* 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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