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Unrepaired Heteroduplex DNA in *Saccharomyces cerevisiae* Is Decreased in *RAD1 RAD52*-Independent Recombination

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ABSTRACT

A direct repeat recombination assay between *SUP4* heteroalleles detects unrepaired heteroduplex DNA (hDNA) as sectored colonies. The frequency of unrepaired heteroduplex is dependent on the mismatch and is highest in a construct that generates C:C or G:G mispairs and lowest in one that generates T:G or C:A mispairs. In addition, unrepaired hDNA increases for all mismatches tested in *pms1* mismatch repair-deficient strains. These results support the notion that hDNA is formed across the *SUP4* repeats during the recombination event and is then subject to mismatch repair. The effects of various repair and recombination defective mutations on this assay were examined. Unrepaired heteroduplex increases significantly only in *rad52* mutant strains. In addition, direct repeat recombination is reduced 2-fold in *rad52* mutant strains, while in *rad51, rad54, rad55* and *rad57* mutants direct repeat recombination is increased 3–4-fold. Mutations in the excision repair gene, *RAD1*, do not affect the frequency of direct repeat recombination. However, the level of unrepaired heteroduplex is slightly decreased in *rad1* mutant strains. Similar to previous studies, *rad1 rad52* double mutants show a synergistic reduction in direct repeat recombination (35-fold). Interestingly, unrepaired heteroduplex is reduced 4-fold in the double mutants. Experiments with shortened repeats suggest that the reduction in unrepaired heteroduplex is due to decreased hDNA tract length in the double mutant strain.

MISMATCH repair is the cellular process used to restore fidelity to DNA after replication errors, base modifications, and heteroduplex DNA (hDNA) formation during recombination. Most models of homologous recombination postulate that during the pairing of DNA molecules, hDNA is formed (HOLLIDAY 1964; MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). hDNA is comprised of one strand of each of the two parental molecules. For example, if the interacting DNA molecules contain base pair differences, then the heteroduplex will contain mismatched base pairs. Correction of the mismatched bases will lead to restoration of either one of the two parental molecules. However, persistence of a mismatch within heteroduplex will result in post-replication segregation of the two alleles. In yeast, post-meiotic segregation (PMS), which gives rise to sectored ascosporal colonies, is interpreted as replicative resolution of hDNA formed during meiotic recombination (reviewed in ORR-WEAVER and SZOSTAK 1985; PETES *et al.* 1991). Indeed, frequencies of PMS display a dependence on the predicted mismatch (WHITE *et al.* 1985). However, a gene conversion event (3:1 or 1:3 segregation) is interpreted as repair of a mismatch in hDNA. LICHTEN *et al.* (1990) have shown that heteroduplex DNA is formed during meiosis. There is also evidence for hDNA formation in mitotic cells undergoing heteroallelic recombination (WILDENBERG 1970; ESPOSITO 1978; GOLIN and ESPOSITO 1981; ROMAN and FABRE 1983), plasmid integration (ROTHSTEIN 1984; ORR-WEAVER *et al.* 1988), and intrachromosomal recombination between non-tandem direct repeat sequences (RONNE and ROTHSTEIN 1988). In addition, recombination frequencies at the *CYC1* locus are significantly affected by different heteroalleles indicating that hDNA is formed and subsequent treatment of mismatched bases can influence mitotic recombination (MOORE *et al.* 1988).

The mechanisms of mismatch repair have been extensively characterized in prokaryotes (reviewed in CLAVERYS and LACKS 1986; RADMAN and WAGNER 1986; MODRICH 1987). However, in yeast, the study of mismatch repair mechanisms is in an earlier stage. Similar to prokaryotes, different single base pair mismatches in yeast are repaired with different efficiencies (BISHOP and KOLODNER 1986; MUSTER-NASSAL and KOLODNER 1986; BISHOP *et al.* 1987; BISHOP *et al.* 1989; KRAMER *et al.*, 1989a). One yeast mismatch repair gene that has been well characterized is *PMS1* (WILLIAMSON *et al.* 1985). Mutations in *PMS1* increase post-meiotic segregation, increase mitotic heteroallelic recombination, cause a mitotic mutator phenotype and decrease the repair of heteroduplex plasmids, suggesting that *pms1* mutant strains are deficient in hDNA correction both during mitosis and meiosis (WILLIAMSON *et al.* 1985; BISHOP *et al.*, 1987; BISHOP *et al.* 1989; KRAMER *et al.* 1989a). *PMS1* shares homology with both the *Escherichia coli MutL* and *Streptomyces pneumoniae HexB* genes

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(Kramer *et al.* 1989b; Mankovich *et al.* 1989; Prudhomme *et al.* 1989). Recently, *MutS* homologues have been identified in yeast (*MSH1-MSH4*) (Valle *et al.* 1991; Reenan and Kolodner 1992a,b; New *et al.* 1993; P. Ross-Macdonald and G. S. Roeder, personal communication). The identification of *Mut* homologs in yeast and more recently man (Fishel *et al.* 1993; Leach *et al.* 1993) indicates that many aspects of mismatch repair have been conserved throughout evolution.

Many radiation-sensitive mutations isolated in yeast have been utilized to understand the mechanisms of recombination and repair (Game and Mortimer 1974; reviewed in Haynes and Kunz 1981; Kunz and Haynes 1981; Friedberg *et al.* 1991; Petes *et al.* 1991). These DNA repair mutants have been categorized into three epistasis groups: *RAD3*, *RAD6* and *RAD52* (Game and Mortimer 1974; reviewed in Haynes and Kunz 1981; Kunz and Haynes 1981; Friedberg 1988; Friedberg *et al.* 1991). *RAD52* group mutants are sensitive primarily to ionizing radiation and mutants in four loci, *rad51*, *rad52*, *rad54* and *rad57* are unable to repair DNA double-strand breaks (Game and Mortimer 1974; reviewed in Haynes and Kunz 1981; Friedberg 1988). *rad52* mutant cells are impaired for HO-catalyzed mating-type interconversion (Malone and Esposito 1980; Weiffenbach and Haber 1981), mitotic gene conversion (Prakash *et al.* 1980; Hoekstra *et al.* 1986; Rothstein *et al.* 1987), direct repeat recombination (Jackson and Fink 1981; Klein and Petes 1981; Willis and Klein 1987; Klein 1988; Schiestl and Prakash 1988; Thomas and Rothstein 1989b), and meiotic recombination (Game *et al.* 1980; Malone and Esposito 1980; Prakash *et al.* 1980). *RAD50* is known to be required for an early step in meiotic recombination (Malone and Esposito 1981; Malone 1983; Borts *et al.* 1986) and for chromosomal synapsis (Malone *et al.* 1985; Alani *et al.* 1990). However, *RAD50* is not required for either inter- or intrachromosomal spontaneous mitotic recombination (Wagstaff *et al.* 1986; Gottlieb *et al.* 1989).

Little is known concerning the functions or roles of the *RAD51*, *RAD54*, *RAD55* and *RAD57* genes in mitotic recombination. Mutations in any of these four *RAD* genes have previously been shown to reduce mitotic heteroallelic recombination in diploids (Saeki *et al.* 1980). In addition, *rad51*, *rad55* and *rad57* strains all show defects in meiotic recombination (Game and Mortimer 1974; reviewed in Haynes and Kunz 1981; Kunz and Haynes 1981; Friedberg 1988; Petes and Symington 1991; Lovett and Mortimer 1987; Shinohara *et al.* 1992). Recently, it has been shown that the Rad51 protein shares significant homology with the bacterial RecA protein and also forms a RecA-like nucleoprotein filament on DNA (Aboussekhra *et al.* 1992; Basile *et al.* 1992; Shinohara *et al.* 1992; Ogawa *et al.* 1993). Biochemical and genetic evidence suggests that the Rad51 protein forms a protein complex with Rad52 (Shinohara *et al.* 1992; Milne and Weaver 1993; F. Fabre personal communication; Bendixen and Rothstein, unpublished observation). In addition, both the *RAD55* and *RAD57* genes contain limited homology to *RecA* (Kans and Mortimer 1991; S. Lovett, personal communication).

The *RAD1* gene is involved in the nucleotide excision repair pathway for ultraviolet radiation damage (reviewed in Haynes and Kunz 1981; Kunz and Haynes 1981; Friedberg 1988; Friedberg *et al.* 1991). Studies from several laboratories suggest that *RAD1* has a role in recombination between direct repeats (Klein 1988; Schiestl and Prakash 1988; Thomas and Rothstein 1989b). It was shown that *rad1* mutants are reduced for some but not all plasmid loss recombination between duplicated genes. Also, it has been suggested that *RAD1* is responsible for *RAD52*-independent gene conversion events (Klein 1988). Moreover, strains mutant for both *rad1* and *rad52* are synergistically reduced for direct repeat recombination (Klein 1988; Schiestl and Prakash 1988; Thomas and Rothstein 1989b). This synergistic interaction suggests that these two genes define alternate pathways for recombination.

Here we utilize a direct repeat recombination assay that detects unrepaired heteroduplex as sectored colonies. We show that the frequency of sectored colony formation is dependent on the nucleotides in the mismatches and is increased in *pms1* mutant strains. Null mutations in several DNA repair and recombination genes were surveyed for their effects on these processes. Of the *RAD52* group, only *rad52* mutants are altered in their frequency of unrepaired heteroduplex. In addition, *rad52* mutant strains exhibit a 2-fold reduction in the frequency of direct repeat recombination, while *rad51*, *rad54*, *rad55* and *rad57* mutants all show a 3–4-fold increased recombination frequency. Strains carrying a disruption of *RAD1* exhibit a decrease in the percent of sectored colonies but do not reduce the recombination frequency. However, the interaction of a *rad1* and *rad52* mutation in the double mutant strain reduces the recombination frequency 35-fold and unrepaired hDNA 4-fold. Using repeats at *SUP4* that differ in the extent of homology, we demonstrated that this reduction in unrepaired heteroduplex in *rad1 rad52* double mutant strains may be due to a decrease in heteroduplex tract length.

MATERIALS AND METHODS

Media: All *S. cerevisiae* strains were maintained on YPD (2% peptone, 2% dextrose, 1% yeast extract). Synthetic complete medium and single omission media used for selection of diploids and for tetrad analysis were made as described previously (Sherman *et al.* 1986) and modified by the addition of 100 µg/ml L-leucine. OFAC medium, used for the *ade2-1* color assay, is synthetic complete medium lacking arginine and containing 1/5 the usual amount of adenine sulfate (4 µg/ml) and 60 µg/ml L-canavanine. 5-FOA medium is synthetic complete medium with 750 µg/ml 5-fluoro-orotic acid and 50 µg/ml uracil (Boeke *et al.* 1984).

TABLE 1

S. cerevisiae strains

Strains	Genotype	Origin
W303-1B	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	ROTHSTEIN laboratory
U626	*MATα SUP4-o::HIS3*	This study
J473	*MATα can1-100,x SUP4-o::HIS3*	This study
U627	*MATα can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI)*	This study
U665	*MATα can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(NheI)*	This study
U666	*MATα can1-100,x SUP4-o::HIS3::pWJ381-CAN1-URA3(MluI)*	This study
U667	*MATα can1-100,x SUP4-o::HIS3::pWJ381-CAN1-URA3(NheI)*	This study
U668	*MATα can1-100,x SUP4-o::HIS3::pWJ382-CAN1-URA3(MluI)*	This study
U669	*MATα can1-100,x SUP4-o::HIS3::pWJ382-CAN1-URA3(NheI)*	This study
W790	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) rad1::LEU2 rad 52-8::TRP1*	This study
W825	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) rad54::LEU2*	This study
W826	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) rad55::LEU2*	This study
W827	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) rad57::LEU2*	This study
W828	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) rad50::hisG*	This study
W838	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) rad1::LEU2*	This study
W838	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) rad52-8::TRP1*	This study
W840	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) pms1::LEU2*	This study
W859	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(NheI) pms1::LEU2*	This study
W860	*can1-100,x SUP4-o::HIS3::pWJ381-CAN1-URA3(MluI) pms1::LEU2*	This study
W861	*can1-100,x SUP4-o::HIS3::pWJ381-CAN1-URA3(NheI) pms1::LEU2*	This study
W862	*can1-100,x SUP4-o::HIS3::pWJ382-CAN1-URA3(MluI) pms1::LEU2*	This study
W863	*can1-100,x SUP4-o::HIS3::pWJ382-CAN1-URA3(NheI) pms1::LEU2*	This study
W881	*can1-100,x SUP4-o::HIS3::pWJ317-CAN1-URA3(MluI) rad51::LEU2*	This study
J589	*MATα can1-100,x sup4::HIS3*	This study
U745	*MATα can1-100,x SUP4-o::HIS3::pWJ445-CAN1-URA3(SmaI)*	This study
W1007	*can1-100,x SUP4-o::HIS3::pWJ445-CAN1-URA3(SmaI)*	This study
W1007	*can1-100,x SUP4-o::HIS3::pWJ445-CAN1-URA3(SmaI) rad1::LEU2 rad52-8::TRP1*	This study
W1090	*TRP1 SUP4-o::URA3*	This study

All strains are isogenic to W303-1B. The genotypes are identical to W303-1B except where noted. Strains designated with a W represent haploid segregants from genetic crosses. In most instances multiple haploid segregants with identical genotypes (except mating type) were used from each cross. However the W number and relevant genotypes, excluding mating type, are listed only once to designate all segregants used.

Strains: All yeast strains used in this investigation were derived from strains W303-1A or W303-1B (THOMAS and ROTHSTEIN 1989a) and are listed in Table 1. Methods for mating, diploid selection, sporulation and tetrad dissection have been described previously (SHERMAN *et al.* 1986). *E. coli* strain SF8 (CAMERON *et al.* 1975) was used as the host for all plasmids.

The plasmid pWJ118 contains a 10.3-kb *Eco*RI to *Bam*HI fragment of the *SUP4-o* locus cloned into pBR322 (ROTHSTEIN *et al.* 1987). Additionally, the *HIS3* marker (STRUHL *et al.* 1979) is inserted in the *Hin*dIII site located approximately 800 bp upstream of *SUP4* (ROTHSTEIN, HELMS and ROSENBERG 1987). The *SUP4-o* gene was introduced into W303-1B by transforming with the plasmid pWJ118 after *Eco*RI-*Bam*HI digestion and selecting for the *HIS3* marker (ROTHSTEIN 1983). A transformant, U626, was used for further studies. U626 is an adenine prototroph and is canavanine sensitive due to the suppression of the *ade2-1* and *can1-100* alleles respectively by *SUP4-o*. A genomic blot (not shown) confirmed the structure of this transformant (SOUTHERN 1975). Similarly, genomic blots were performed to confirm all of the constructs described below. To permit complementation by the wild-type *CAN1* gene (see below), canavanine resistant mutants that contain a second non-suppressible mutation in the *can1-100* allele (*can1-100,x*) were selected on canavanine-containing medium (60 µg/ml). These mutants were tested for their ability to grow on adenine-free medium to insure that the suppressor was still functional. One such strain, J473, was used for integration of the plasmids pWJ317, pWJ381 and pWJ382 to create the *SUP4* duplications (see below).

Disruptions of the *RAD* genes and the *PMS1* gene were introduced into the genome by transforming linearized DNA (ROTHSTEIN 1983) into yeast made competent by the LiCl method (ITO *et al.* 1983). The presence of the *rad* and *pms1* disruptions were confirmed by analysis of genomic DNA of transformants by DNA hybridization blots (SOUTHERN 1975). Additionally, the *rad* disruptions were verified by sensitivity of the mutant strain to ionizing radiation (25 krad, in a cesium source emitting 7.8 krad/h) and by their genetic linkage to known mutations. The *rad52*, *rad54*, *rad55* and *rad57* disruption plasmids were kindly provided by DAVID SCHILD (SCHILD *et al.*, 1983a,b). The *rad50 hisG-URA3-hisG* disruption plasmid was kindly provided by NANCY KLECKNER (ALANI *et al.* 1987). Loss of the *URA3* gene between the *hisG* direct repeats was selected on 5-FOA medium to create the *rad50::hisG* disruption used in these experiments. The *rad51* disruption plasmid was kindly provided by LORRAINE SYMINGTON. The *rad1::LEU2* disruption was previously constructed in this laboratory in an isogenic genetic background (RONNE and ROTHSTEIN 1988). A *pms1* deletion plasmid was kindly provided by WILFRED and BARBARA KRAMER (KRAMER *et al.* 1989b). A *LEU2* selectable marker was inserted at a unique *Sac*I site within the remaining sequences of the *pms1* deletion to facilitate transformation and subsequent genetic analysis of the allele. Segregants containing single or multiple *rad* mutations were made by crossing the appropriate disrupted strains to previously constructed isogenic strains containing the *SUP4-o::pWJ317-CAN1-URA3::sup4* plasmid duplication (described below).

Duplications: Six different suppressor duplications were used to monitor mitotic recombination and investigate the effect that different alleles have on the formation and repair of heteroduplex DNA. The plasmids used to create these duplications are YIp5 based (STRUHL *et al.* 1979) and contain a 2.3-kb *Hin*dIII/*Sal*I insert from the *SUP4* region as well as a 3.9-kb *Xho*I fragment of the *CAN1* gene inserted at the *Sal*I site (BROACH *et al.* 1979; HOFFMANN 1985). The plasmid pWJ317 contains the wild-type *sup4* $tRNA^{Tyr}$ gene (Figure 1A). pWJ381

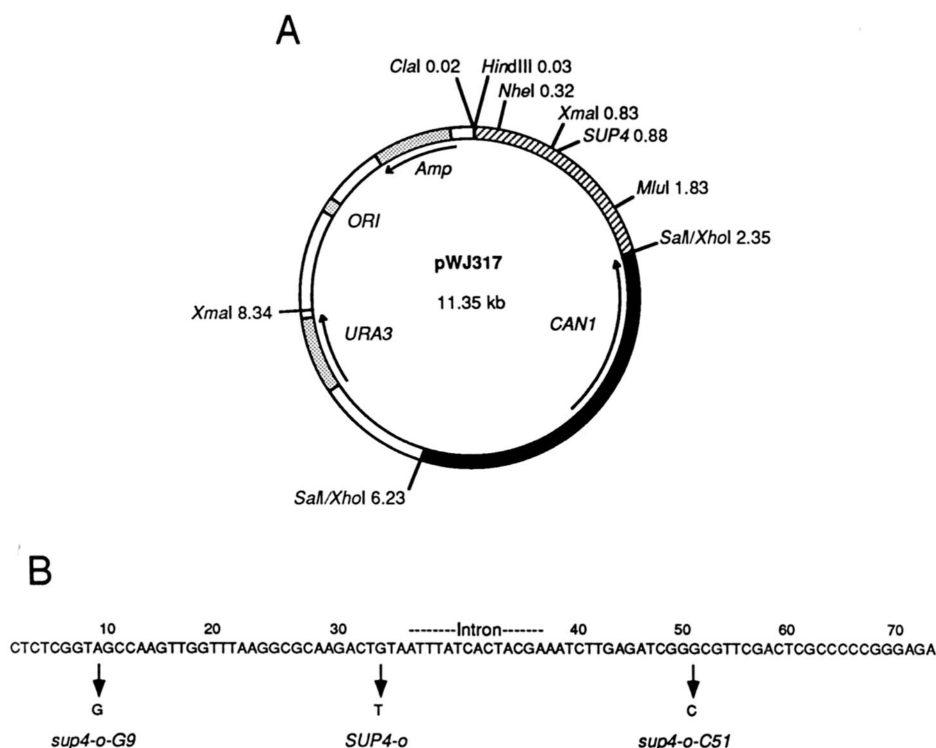


FIGURE 1.—Plasmid pWJ317 used to create *SUP4* duplications and the *sup4*sequence. (A) pWJ317 is a derivative of YIp5 with the yeast *SUP4* chromosomal locus from *Hin*dIII to *Sal*I cloned into the *Hin*dIII and *Sal*I sites of YIp5 (▨). In addition, a *Xho*I to *Xho*I yeast chromosomal fragment containing the *CAN1* gene is cloned into the *Sal*I site ■. The ampicillin resistance gene (*Amp*), the plasmid origin of replication (*ORI*) and the yeast *URA3* gene are also indicated. The numbers after the restriction sites refer to nucleotide positions relative to the *Eco*RI site of YIp5. This plasmid and derivatives of it were integrated into the yeast genome by digestion with either *Nhe*I or *Mlu*I to create chromosomal duplications at *SUP4*. (B) The sequence of the wild-type *sup4* gene and the positions of the suppressor anticodon and second-site mutations. The *sup4*-**o**-*G9* mutation creates a new *Bal*I site and the *sup4*-**o**-*C51* mutation creates a new *Eag*I site. The nucleotide positions are numbered according to the system of SPRINZL *et al.* (1980).

and pWJ382 are essentially identical to pWJ317 except each contain a *SUP4*-**o** gene that harbors either an A to G transition at position nine or a G to C transversion at position 51, respectively, within the gene (nucleotide positions are numbered according to the system of SPRINZL *et al.* (1980)) (Figure 1B). These second-site mutations were selected by virtue of their inactivating effect on the suppressor (KURJAN and HALL 1980). All of the DNA segments containing *SUP4*-**o** or second-site mutations were derived from isogenic *SUP4* regions from the strain 164RW87 (ROTHSTEIN *et al.* 1977; KURJAN and HALL 1980).

To create the *SUP4* duplications, the plasmids were digested separately with a restriction enzyme (*Mlu*I or *Nhe*I) that maps uniquely on each side of the anticodon (Figure 1A) to target them to the *SUP4* locus of strain J473, which carries the *HIS3* marker approximately 800 bp upstream of *SUP4* (ORR-WEAVER *et al.* 1981). In this manner, the strains carrying both orientations of the alleles were constructed (Table 1 and Figure 2). The orientation of the *SUP4* alleles was confirmed by genomic blot analysis and/or allele rescue experiments.

An additional duplication containing a shortened region of *SUP4*-**o** was created using a construct essentially identical to pWJ317 that contains an approximately 880-bp region of *SUP4*-**o**. The plasmid was constructed as follows. An *Nhe*I-*Nde*I *SUP4*-**o** fragment was cloned into the *Sph*I site in the polylinker of pUC19 after the addition of *Sph*I linkers (d(pG-GCATGCC)). Digestion with *Hin*dIII and *Sal*I releases a *SUP4* fragment that retains short polylinker sequences. *Mlu*I linkers (d(pGACGCGTC)) were added at the *Hin*dIII site of this *SUP4* fragment and at the *Cla*I site of pWJ317 facilitating the replacement of the 2.3-kb *SUP4* region (from *Cla*I to *Sal*I) with an approximately 880-bp *SUP4* region. The *Xma*I site outside the *URA3* gene in pWJ317 was destroyed after digestion with *Xma*I, filling in the overhang with T4 DNA polymerase followed by blunt-end ligation. This scheme yields a unique *Xma*I (or *Sma*I) recognition sequence in the *SUP4* gene. Integration of this plasmid (pWJ445) at *SUP4* was achieved by linearizing the plasmid at the *Sma*I or *Xma*I restriction site and transforming into strain J589 (ORR-WEAVER *et al.* 1981).

***SUP4* direct repeat recombination assay:** As described previously (RONNE and ROTHSTEIN 1988), cells containing *SUP4* duplications were streaked on medium lacking adenine and uracil (−Ade −Ura) to select against mitotic recombination events prior to plating on OFAC medium. For example, cells that have undergone a recombination event such that the intervening plasmid sequences are excised (referred to as plasmid excision, plasmid loss or popout events) require uracil and therefore fail to grow. In addition, recombination events resulting in two wild-type tRNA genes require adenine and fail to grow. Recombination events resulting in two suppressors are lethal in a haploid strain and therefore are eliminated (GILMORE and MORTIMER 1966). Single colonies were next inoculated into 5 ml of −Ade −Ura liquid medium and grown to a density of 2–5 × 10^7 cell/ml. The cultures were sonicated for 2.5 min (Artek Sonic 300 Dismembrator set at 35) to ensure the plating of single cell colony forming units. Next, the cultures were counted under a hemocytometer, diluted with sterile distilled H_2O and an appropriate volume of cells were plated to OFAC medium to select for canavanine resistant recombinant colonies and to synthetic complete medium to determine the number of viable plating units. Recombinant colonies were next scored for their color: either red, white or sectored. For strains carrying the *rad1::LEU2*, *rad52-8::TRP1* or the *pms1::LEU2* mutation, the OFAC plates were replica plated to −Ura medium to detect canavanine resistant Ura^+ colonies that are due to mutation in the *CAN1* gene and not to recombination. Strains carrying both the *rad1* and *rad52* mutations give rise to a high background of canavanine resistant Ura^+ colonies. Therefore, the OFAC plates were replica plated to 5-FOA to score truly recombinant colonies that are both Can^r and Ura^-. In *rad51*, *rad54*, *rad55* and *rad57* strains

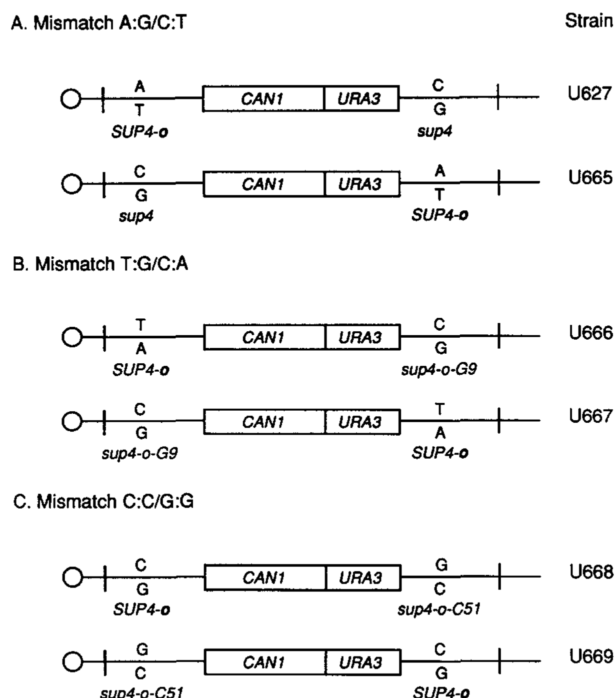


FIGURE 2.—Duplications at *SUP4*. The duplications consist of an approximate 2.3-kb region of the *SUP4* locus with 1.5 kb of homology to the right of *SUP4* and 880 bp of homology to the left. The *HIS3* marker (not shown) is located approximately 800 bp upstream of the *SUP4* repeats. The repeated sequences are shown bordered by vertical lines and the *CAN1* or *URA3* markers. Differences in base composition between the repeats for each duplication and position of *SUP4* are indicated. Integration of plasmids by linearizing with either *Mlu*I or *Nhe*I creates chromosomal duplications in both orientations of the *SUP4* repeats. (A) Integrations of a *sup4*-containing plasmid (pWJ317). (B) Integrations of a *sup4*-**o**-*G9*-containing plasmid with a second-site mutation A to G transition at nucleotide 9 (pWJ381). (C) Integrations of a *sup4*-**o**-*C51*-containing plasmid with a second-site mutation G to C transversion at nucleotide 51 (pWJ382).

the level of non-recombinant Can^r and Ura$^-$ colonies does not contribute significantly to the frequency of canavanine resistance. Although, the *SUP4* region can undergo delta-mediated deletions, these events occur at a frequency 3 orders of magnitude lower than direct repeat recombination and therefore do not contribute significantly to canavanine resistant events (ROTHSTEIN *et al.* 1987).

Statistical analysis: Six to nine trials for each strain were performed. Since cells are grown selectively prior to plating on OFAC, recombination frequencies are highly reproducible and closely approximate the rate of recombination. The recombination frequencies are reported as mean values. Contingency table chi-squared analysis was used to determine whether the percent sectored colonies and recombination frequencies were significantly different between strains.

Segregation analysis of white sectors: White sectors from red/white sectored recombinant colonies from *pms1* and *rad52* strains were picked at random and back crossed to W1090-2A or W1090-4B in which the *URA3* marker is linked to *SUP4*-**o** (*ade2-1 SUP4*-**o**::*URA3*; Table 1). Recall that *HIS3* is linked to all *SUP4* duplication strains analyzed (*SUP4::HIS3*; Table 1). The diploids were sporulated and 6 tetrads from each diploid were dissected. The dissection plates were replica plated to omission media to follow the Ade, His, Leu, Trp and Ura phenotypes.

This analysis was performed to determine whether the white sectors were genotypically *SUP4*-**o** or contain a novel mutation that arose within a red *sup4* recombinant colony giving rise to a white sector. Examples of such mutations include: ochre suppressors other than *SUP4*-**o**, reversion of *ade2-1* or mutations in other adenine biosynthetic genes. Meiotic segregation can be used to distinguish among these possibilities. Segregation of the *SUP4*-**o**::*HIS3* allele from the *SUP4*-**o**::*URA3* allele will result in a 2:2 segregation of the His$^+$ from Ura$^+$ phenotypes and 4:0 Ade$^+$ to Ade$^-$ segregation. Segregation of a novel ochre suppressor in a *sup4::HIS3* strain crossed to the *SUP4*-**o**::*URA3* strain would result in lethality when the novel suppressor segregates into the same spore with *SUP4*-**o**::*URA3*, since two suppressors in a haploid is lethal (*e.g.*, nonparental: 2 alive Ade$^-$ His$^+$ to 2 dead spores; tetratype: 2 alive Ade$^+$ spores, 1 alive Ade$^-$ His$^+$ spore and 1 dead spore). Segregation of a mutation in a gene in the adenine biosynthetic pathway would result in both red and white Ade$^-$ His$^+$ spore colonies. Finally, segregation of a reversion of *ade2-1* (*ADE2*) from *sup4::HIS3* would result in Ade$^-$ His$^+$ spore colonies.

RESULTS

A visual assay for detection of unrepaired heteroduplex: RONNE and ROTHSTEIN (1988) provided evidence that heteroduplex DNA forms during direct repeat recombination. They noted that, although unlikely, sectored colonies could arise by means other than unrepaired hDNA, such as a concerted G_2 event. For example, a single DNA lesion that occurred in the G_1 phase of the cell cycle could stimulate two concerted events after replication, giving rise to a sectored colony. To strengthen the argument that sectored colonies reflect a hDNA intermediate, we designed experiments to demonstrate that the formation of sectored colonies is correlated with the efficiency of mismatch repair. Work both in bacteria and yeast suggests that different mismatched base pairs are corrected with different efficiencies (reviewed in CLAVERYS and LACKS 1986; RADMAN and WAGNER 1986; MODRICH 1987; SMITH 1988). Therefore, repeats with different single base pair changes were analyzed to observe any differences in the percent of sectored colonies. In addition, strains carrying a known mismatch repair defective mutation, *pms1*, were also analyzed for the effect on the frequency of sectored colonies.

Plasmids containing the arginine permease gene, *CAN1*, the *URA3* gene, and the *sup4* tRNA gene or a *SUP4*-**o** suppressor carrying an inactivating second-site point mutation, *sup4*-**o**-*x* (Figure 1B), were integrated at the *SUP4*-**o** locus as described in MATERIALS AND METHODS. This results in a duplication of the *SUP4* region with each repeat differing by a single base pair (Figure 2). In our previous study we found that selection on 5-FOA medium for the loss of the *URA3* gene results in a phenotypic lag requiring an elaborate filter transfer method to visualize sectored colonies (RONNE and ROTHSTEIN 1988). To simplify the assay, we have used the *CAN1* marker, which displays no phenotypic lag, to select directly for plasmid loss. Direct repeat re-

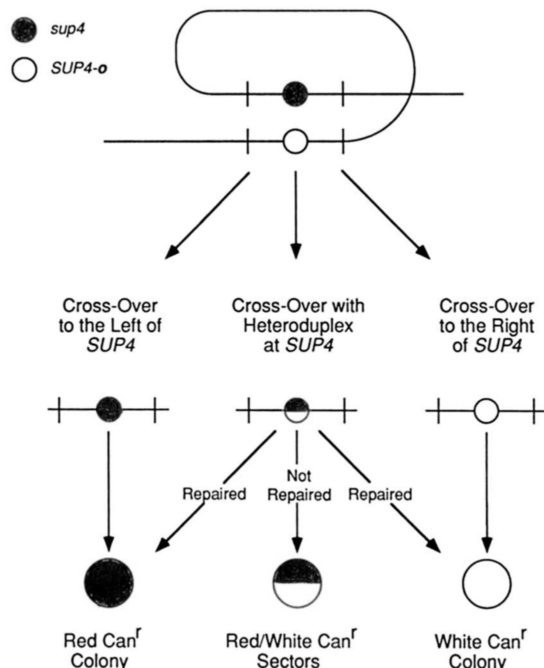


FIGURE 3.—Red/white assay for events associated with plasmid loss. Only one possible interaction of the repeats is pictured (intrachromosomal recombination). The open circle ○ refers to white *SUP4-o* recombinant colonies. The black circle ● refers to red *sup4* recombinant colonies. Three possible outcomes of the recombination event are pictured. Crossovers to right of *SUP4* result in white colonies, while crossovers to the left of *SUP4* result in red colonies. If during recombination heteroduplex forms across *SUP4* then a mismatch will be generated indicated by a half open and half black circle. A sectored colony is indicative of an unrepaired mismatch within heteroduplex.

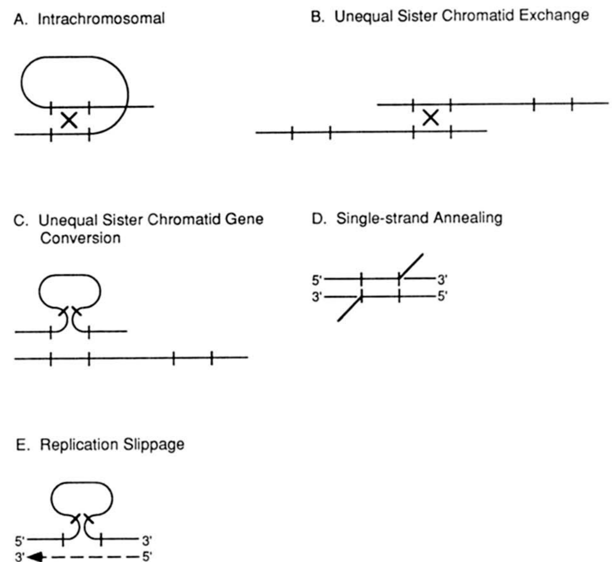


FIGURE 4.—Possible mechanisms of plasmid loss recombination. (A) Intra-chromosomal recombination proceeds when the repeated sequences on the same chromosome interact. The intervening plasmid DNA is "looped-out" or deleted from the chromosome. (B) Unequal sister chromatid exchange occurs in the G_2 phase of the cell cycle and repeated sequences from sister chromatids pair in an unequal fashion. A crossover will result in a triplication on one of the sisters. On the other sister chromatid, only a single copy of the repeat remains; thus no intervening plasmid DNA remains. (C) Similar to unequal sister chromatid exchange, unequal sister chromatid gene conversion occurs in G_2. However, both the repeated sequences on one sister pair with one of the repeated sequences on the other sister. The intervening DNA is lost as a result of the conversion event. (D) Single-strand annealing occurs when the 5′ DNA ends at a site of a double-strand break are degraded removing the intervening DNA. When a region of homology is exposed, the homologous regions anneal to form a joint molecule to repair the break. Duplex DNA is drawn showing both DNA strands. (E) Replication slippage occurs during DNA synthesis. The DNA polymerase and the nascent replicated strand realign from one repeat to the other. Replication resumes without replicating the intervening DNA. Template DNA is shown as a continuous line, while the nascent strand is shown as a dashed line.

combination results in the loss of intervening DNA, leaving only a single copy of the repeat. Demonstration that a canavanine resistant colony is concomitantly Ura$^-$ confirms the loss of the intervening DNA. In addition, the ochre suppressible allele, *ade2-1*, permits a visual scoring of the *SUP4* genotype. In the presence of *SUP4-***o**, colonies are white, in its absence, they are red. Depending on the position of the crossover, either a red or a white Can^r colony is obtained (see Figure 3). However, if hDNA is formed across the *SUP4* locus during the recombination event and is unrepaired, a red/white sectored colony will be formed (Figure 3). Although several models can be devised for the mechanism of plasmid loss (Figure 4), for each model a sectored colony is taken as evidence that hDNA was the intermediate during the recombination event. Furthermore, the percentage of sectored colonies is determined not only by the frequency of hDNA formation but also by the efficiency of mismatch correction (Figure 3).

Sectored colony formation is dependent on the specific mismatch: Several alleles of *SUP4* were tested to determine their effect on the formation of sectored colonies. The alleles were chosen on the basis of the predicted repair efficiencies of the mismatches that would result from the formation of hDNA (MUSTER-NASSAL and KOLODNER 1986). The two potential mismatches created during a recombination event between the *SUP4-***o***/sup4* repeats are A:G/C:T (Figure 2). Using sequenced isogenic second-site point mutations that inactivate the *SUP4-***o** gene (KURJAN and HALL 1980) (Figure 1B), duplications that potentially result in C:C/G:G or T:G/C:A mismatches were also analyzed (Figure 2). Mean frequencies of direct repeat recombination were determined from six to nine independent trials (Table 2). The frequency of plasmid excision was unaffected by the different heteroalleles of *SUP4* (1.1–1.4×10^{-4}). In addition, the percent of sectored recombinant colonies was determined for each of the duplication strains (Table 2). The T:G/C:A transition

TABLE 2

Recombination and sectored colony formation in wild-type and *pms1* mutant strains

		Wild-type		*pms1*	
Strain[a]	Potential mismatches[b]	Percent sectored colonies[c]	Recombination frequency[d] Can^r ($\times 10^{-4}$)	Percent sectored colonies[c]	Recombination frequency[d] Can^r ($\times 10^{-4}$)
U627	A:G/C:T	9.7 (298/3064)	1.4 ± 0.3	20.3 (363/1790)	1.4 ± 0.4
U665	A:G/C:T	8.9 (286/3207)	1.3 ± 0.3	20.4 (501/2454)	1.4 ± 0.2
U666	T:G/C:A	4.7 (113/2406)	1.2 ± 0.3	12.7 (491/3854)	1.9 ± 0.4
U667	T:G/C:A	3.6 (92/2527)	1.1 ± 0.2	11.1 (202/1823)	0.9 ± 0.2
U668	C:C/G:G	13.1 (474/3615)	1.4 ± 0.3	15.8 (347/2191)	1.3 ± 0.2
U669	C:C/G:G	11.2 (354/3138)	1.2 ± 0.2	23.1 (528/2283)	1.2 ± 0.3

[a] Strain name refers to the wild-type strains. The corresponding *pms1* strain names are listed in Table 1.
[b] Refers to the mismatches that can form within a heteroduplex.
[c] The percentage of sectored colonies was determined by dividing the total number of sectored recombinant colonies by the total number of recombinant colonies recovered from six to nine independent trials. These numbers are indicated in parentheses. Chi-squared analysis was performed to determine significance. The percent sectored colonies for all strains carrying the *pms1::LEU2* allele was different from the corresponding wild-type strains at the $P \leq 0.01$ level.
[d] The recombination frequency is the mean value from six to nine independent trials and was determined by dividing the total number of recombinant colonies recovered by the total number of viable cells plated. As described in MATERIALS AND METHODS, non-recombinant Can^rUra^+ colonies from *pms1* strains were eliminated from the analysis. Standard deviations are $\sigma n - 1$ values. Chi-squared analysis was performed to determine significance. The recombination frequency for all strains carrying the *pms1::LEU2* allele was not different from the corresponding wild-type strains at the $P \leq 0.05$ level.

mismatches exhibit the fewest sectored colonies (3.6–4.7%), the C:C/G:G transversion mismatches exhibit the highest percentage (11.2–13.1%), and the A:G/C:T mismatches exhibit an intermediate level (8.9–9.7%). These results are consistent with the results of MUSTER-NASSAL and KOLODNER (1986) and BISHOP *et al.* (1989) and support the idea that the frequency of sectors is mismatch dependent.

Sectored colony formation increases in *pms1* mutant strains: Further evidence that the frequency of sectored colonies reflects unrepaired hDNA derives from studies involving the mismatch repair deficient mutation *pms1*. We introduced a disruption of the *PMS1* gene into our direct repeat recombination assay strains. Contrary to what is observed for heteroallelic recombination between homologs (WILLIAMSON *et al.* 1985), the *pms1* mutation does not increase the frequency of direct repeat recombination in our assay (Table 2). The percentage of sectored colony formation after these events increases between 2- and 3-fold for five of the six duplication strains (U627, U665, U666, U667 and U669) (Table 2). In the remaining strain, U668, a smaller but statistically significant increase is observed. These results are consistent with previous findings that heteroduplex repair is impaired in *pms1* mutant strains (BISHOP *et al.* 1989; KRAMER *et al.* 1989a). Further, these results strongly support the argument that sectored colonies reflect unrepaired mismatches that arise within heteroduplex DNA during direct repeat recombination.

Mutations in *RAD52* group genes comprise three classes with respect to direct repeat recombination: Null mutations in several *RAD52* group genes were created in an isogenic strain background (W303-1B; Table 1) to investigate whether any of these recombination/repair genes affect plasmid excision recombination or the formation of sectored colonies. All *rad* disruption strains contain the U627 duplication (Figure 2). Table 3 shows that *rad52* strains exhibit an increase in unrepaired heteroduplex over wild-type levels (15.5%, $P \leq 0.01$). This increase in sectored colonies differs from our previous report where we found no significant difference between wild-type and *rad52* strains (RONNE and ROTHSTEIN 1988). However, the discrepancy can be explained by the increased mutation rate in *rad52* strains (VON BORSTEL *et al.* 1971; KUNZ *et al.* 1989). In the previous report, there was no means of distinguishing between true plasmid excision events and colonies that had a mutation in the counter selectable marker (*URA3*). In the present study, Can^r colonies that remain Ura^+ (due to mutations in the *CAN1* marker) are easily identified by replica plating to medium lacking uracil.

We found that the percentage of sectored colonies in *rad50*, *rad51*, *rad54*, *rad55* and *rad57* disruption strains did not differ greatly from wild type (Table 3), suggesting that the extent of heteroduplex DNA formation and mismatch repair at *SUP4* is not significantly altered in any of these mutants. Further, the level of recombination in these mutant strains fell into two classes: wild-type levels (*rad50*) and 3–4-fold elevated (hyper-rec) (*rad51*, *rad54*, *rad55* and *rad57*). Therefore, there are three phenotypic classes within the *RAD52* epistasis group with respect to direct repeat recombination.

Increased percent of sectored colonies in *pms1* and *rad52* strains is not due to increased mutation: Both the *pms1* and *rad52* mutations are known to increase the spontaneous mutation rate (WILLIAMSON *et al.* 1985; KUNZ *et al.* 1989). In our strains, disruption of the *PMS1*

TABLE 3

The effect of *rad* mutations on recombination at *SUP4*

Strain[a]	Percent sectored colonies[b]	Recombination frequency[c] Can^r ($\times 10^{-4}$)	Relative frequency[d]
wt	9.7 (298/3064)	1.4 ± 0.3	1
rad1	8.4 (547/6494)	1.7 ± 0.3*	1
rad50	8.6 (181/2103	1.2 ± 0.2*	1
rad51	10.4 (1018/9752)	5.9 ± 0.7	4
rad52	15.5 (459/2956)	0.7 ± 0.1	0.5
rad54	9.5 (584/6121)	4.4 ± 0.6	3
rad55	10.0 (929/9307)	5.4 ± 0.5	4
rad57	9.0 (684/7640)	5.5 ± 0.3	4
rad1 rad52	2.3 (17/736)	0.04 ± 0.01	0.03

[a] Indicates the disrupted *rad* gene. All strains contain the U627 duplication.

[b] The percentage of sectored colonies and significance was determined as described in Table 2 and MATERIALS AND METHODS. The number of sectored colonies per recombinant colonies is indicated within the parentheses. The percent of sectored colonies obtained from *rad1*, *rad52* and *rad1 rad52* mutant strains was different from wild-type at the $P \leq 0.01$ level.

[c] The recombination frequency and significance was determined as described in Table 2 and MATERIALS AND METHODS. As described in MATERIALS AND METHODS, non-recombinant Can^r Ura$^+$ colonies from *rad1*, *rad52* and *rad1 rad52* strains were eliminated from the analysis. All frequencies were different from wild-type at the $P \leq 0.05$ level, except those frequencies indicated by an asterisk (*).

[d] Refers to the fold change of the recombination frequency from that of the wild-type strain.

and *RAD52* genes cause an approximate 10-fold increase in the spontaneous mutation rate at the *CAN1* marker (data not shown). The increased mutation rate displayed by these strains could be responsible for the increased percent of sectored colonies also displayed by these strains. Examples of spontaneous mutations that could result in a white sector within a *sup4::HIS3* red recombinant colony include: ochre suppressors, reversion of *ade2-1* and mutations in other adenine biosynthetic genes. As outlined in MATERIALS AND METHODS, using a segregation analysis, these kinds of events can be distinguished from true *SUP4-o* sectors. Therefore, 27 *rad52* (strain W838) and 60 *pms1* random white sectors (strains W840, W860 and W863) were backcrossed to *SUP4-o::URA3* strains (W1090). Six tetrads from each diploid were dissected and the segregations of the Ade, Ura and His phenotypes were scored. Fifty-eight of 60 *pms1* white sectors contain *SUP4-o* indicating that no more than 1.7% of sectored colonies are due to mutations. Similarly, less than 4% (1 out of 27) *rad52* white sectors result from mutations. Therefore, the nearly 60% increase in the percent of sectored colonies observed in *rad52* strains and the 2–3-fold increase in *pms1* strains is due to unrepaired heteroduplex and not an elevation of the spontaneous mutation rate.

Recombination and sectored colony formation are reduced in *rad1 rad52* double mutant strains: We examined whether a null mutation in the excision repair gene, *RAD1*, has an effect on plasmid excision and on sectored colony formation. Similar to *rad52*, *rad1* mutant cells exhibit a mitotic mutator phenotype (KUNZ *et al.* 1990) that leads to an increase in non-recombinant Can^r colonies. After correcting for such mutations, we found that the frequency of plasmid excision events between the *SUP4* repeats is not significantly altered in *rad1* cells (Table 3). However, the percentage of sectored colonies is slightly reduced in *rad1* mutant strains (8.4%) ($P \leq 0.01$).

We next investigated *rad1 rad52* double mutants, which are known to be synergistically reduced for direct repeat recombination. In our assay, recombination is reduced 35-fold. Moreover, a 4-fold reduction in sectored colony formation is observed (2.3%) (Table 3). Thus, not only is recombination drastically reduced in the double mutant but also the residual recombination is characterized by reduced unrepaired heteroduplex.

Shortened hDNA tracts in *rad1 rad52*-independent recombination: There are at least two explanations for the reduction in unrepaired heteroduplex in *rad1 rad52* strains. Firstly, an increase in the efficiency of mismatch repair could result in fewer sectored colonies due to either an efficient mismatch repair mechanism associated with *rad1 rad52*-independent recombination or an increase in the time available for mismatch repair. Secondly, the length of heteroduplex DNA could be shortened in the double mutant. Since the probability of a nucleotide being included within a region of heteroduplex is dependent on two factors, heteroduplex tract length and the length of the homologous region, shortened hDNA tracts would reduce the probability that the heteroalleles would be included within the region of heteroduplex (Figure 5). If the first hypothesis is correct, then the decrease in sectored colonies observed in *rad1 rad52* strains will be independent of the length of the repeated region. However, if the second hypothesis is correct, then reducing the length of the repeats will increase the percent of sectored colonies in both wild-type and *rad1 rad52* strains (Figure 5).

These hypotheses were tested by analyzing sectored colony formation in strains that carry shortened *SUP4* repeats (approximately 880 bp in length: ~560 bp of homology to the left of *SUP4* and 323 bp to the right, Figure 6). In wild-type strains the frequency of recombination between the 880-bp repeats is 2-fold higher than between the 2.3-kb repeats. Similarly, previous studies have shown that the frequency of recombination between repeated sequences does not always correlate with the length of homology (THOMAS and ROTHSTEIN 1989a; YUAN and KEIL 1990). We found that a 2.6-fold decrease in the length of the *SUP4* repeats results in a 2.2-fold increase in the percent of sectored colonies in wild-type strains (Table 4). In *rad1 rad52* double mutant strains, the percent of sectored colonies is increased 2.3-fold (2.3 to 5.4%) in response to reducing the homology. Therefore, in *rad1 rad52* strains, the reduced

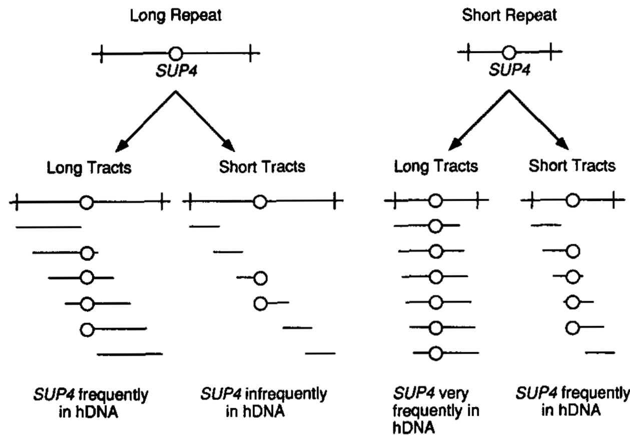


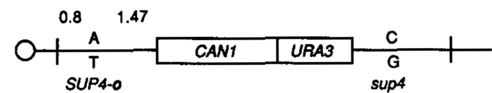
FIGURE 5.—Length of repeat *vs.* length of hDNA. *SUP4* has a certain probability of inclusion in a heteroduplex tract thereby forming mismatched bases and possibly giving rise to a sectored colony. The probability of *SUP4* being included within heteroduplex reflects the length of the heteroduplex tract (*e.g.*, long heteroduplex tracts will frequently include *SUP4* whereas short heteroduplex tracts will less frequently include *SUP4*). Shortening the length of the repeat increases the probability that *SUP4* will be included in a heteroduplex tract.

unrepaired heteroduplex is likely a consequence of shortened tract length (the second hypothesis).

DISCUSSION

We have developed an assay that detects unrepaired heteroduplex DNA during mitotic recombination between non-tandem repeated DNA. Previously, it was shown that sectored colonies could be detected among recombinants (RONNE and ROTHSTEIN 1988). However, it was not definitively shown that sectored colonies were due to unrepaired heteroduplex DNA. Therefore, we showed that sectored colony formation is dependent both on the efficiency of mismatch repair and on *PMS1*, a known mismatch repair gene. Our results indicate that different heteroallelic duplications give rise to different levels of sectored colonies that correspond to the known repair efficiencies for these mispairs. For example, strains that can potentially form T:G/C:A mismatches (U666 and U667) exhibit the lowest percentage of sectored colonies, while strains that can potentially form C:C/G:G mismatches (U668 and U669) exhibit the highest percentage. These observations are consistent with the T:G mismatch being the most efficiently repaired and the C:C mismatch being the least efficiently repaired (MUSTER-NASSAL and KOLODNER 1986; BISHOP *et al.* 1989; KRAMER *et al.* 1989a). In addition, we found that introduction of the *pms1* mutation into the duplication strains causes an increase in the percent of sectored colonies. Our results correspond strikingly well with those of BISHOP *et al.* (1989), who found that unrepaired mismatches after transformation into *pms1* strains increase 1.5–3-fold compared to wild-type levels. Since the formation of sectored colonies in our

A. 2.3 kb repeat



B. 880 bp repeat

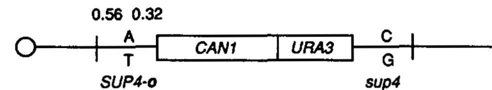


FIGURE 6.—Comparison of different sized repeats at *SUP4*. The 2.3-kb repeat is the U627 duplication (Figure 2). In both duplications, 2.3 kb and 880 bp, the potential mismatches are A:G/C:T. The numbers above each duplication indicate the approximate length of homology to the left and right of *SUP4*.

assay is affected by both the heteroalleles used and the *pms1* mutation, we conclude that the appearance of sectored colonies is due to the post-replicative segregation of unrepaired heteroduplex formed during recombination.

The *SUP4* duplications were constructed in both orientations to examine whether there is asymmetry in the formation of heteroduplex DNA leading preferentially to the generation of one of the two potential mismatches. We found that the U668 duplication in a *pms1* background exhibits less of an increase in percent sectored colonies than the other duplications (Table 2). One interpretation of this result is that hDNA forms asymmetrically, generating preferentially the C:C mispair in U668. This hypothesis is consistent with the finding that the low level of repair of the C:C mismatch is not reduced in *pms1* mutant strains (BISHOP *et al.* 1989; KRAMER *et al.* 1989a). If asymmetry exists, then the G:G mispair, which is repaired 5–9 times more frequently than C:C (BISHOP *et al.* 1989; KRAMER *et al.* 1989a), would preferentially be generated in the U669 duplication (opposite orientation). Since G:G mispairs are more efficiently repaired than C:C, this would predict that the percent of sectored colonies in U669 would be significantly less than in U668. However, there is only a marginal difference between U668 and U669 (Table 2). Therefore, we suggest that there is no asymmetry in heteroduplex formation.

The average length of heteroduplex DNA tracts in wild-type strains can be estimated by comparing the percent of sectored colonies between the 2.3-kb repeats and the 880-bp repeats. This estimation relies on two assumptions but makes no assumption about the specific mechanism (*e.g.*, see Figure 4). First, shortening the length of homology does not dramatically influence the length of heteroduplex tracts. Second, the mechanism(s) of recombination between shorter regions of homology is not different from the mechanism(s) of recombination between longer regions of homology. We postulate that the probability of *SUP4* being in-

TABLE 4

Sectored colonies: dependence on length of homology

Strain [a]	2.3-kb repeats		880-bp repeats	
	Percent sectored colonies [b]	Recombination frequency [c] Can^r ($\times 10^{-4}$)	Percent sectored colonies [b]	Recombination frequency [c] Can^r ($\times 10^{-4}$)
wt	9.7 (298/3064)	1.4 ± 0.3	21.7 (901/4161)	2.7 ± 0.6
rad1 rad52	2.3 (17/736)	0.04 ± 0.01	5.4 (16/294)	0.01 ± 0.004

[a] Indicates the disrupted *rad* gene. Strain names are listed in Table 1.

[b] The percentage of sectored colonies and significance was determined as described in Table 2 and MATERIALS AND METHODS. The number of sectored colonies per recombinant colonies is indicated within the parentheses. The percent sectored colonies for all strains carrying the 880-bp repeats was different from the corresponding strains carrying the 2.3-kb repeats at the $P \leq 0.01$ level. The percent of sectored colonies obtained from *rad1 rad52* mutant strains carrying the 880-bp repeats was different from the corresponding wild-type strains at the $P \leq 0.01$ level.

[c] The recombination frequency and significance was determined as described in Table 2 and MATERIALS AND METHODS. As described in MATERIALS AND METHODS, non-recombinant Can^r Ura$^+$ colonies from *rad1 rad52* strains were eliminated from the analysis. The recombination frequency for *rad1 rad52* strains carrying the 880-bp repeats was different from the corresponding wild-type strains at the $P \leq 0.05$ level.

cluded within a region of heteroduplex increases in a linear fashion as the length of homology between the repeats is decreased, as long as the average length of heteroduplex is equal to or shorter than the repeats. However, at the point where the average length of heteroduplex surpasses the length of homology, no further increase in percent sectored colonies will occur. Sectored colony formation increases only 2.2-fold in wild-type strains (21.7%/9.7%; Table 4) compared to the hypothetical 2.6-fold increase predicted by the change in the length of the repeats (2300 bp/880 bp). This difference is significant ($P \leq 0.01$) and suggests that the length of heteroduplex tracts is slightly longer than the length of the short repeats (880 bp). An estimate of the average tract length of hDNA is made by dividing the length of the long repeats by the fold increase in sectored colonies. Thus, the average length of hDNA is 1045 bp (2300 bp/2.2-fold). Since we estimate that the average length of heteroduplex is longer than the short repeats, when the short repeats recombine *SUP4* should always be included within heteroduplex (~100% heteroduplex). Therefore, the efficiency at which the mismatches are repaired can be calculated by subtracting 21.7% unrepaired hDNA (Table 4) from 100% hDNA. This level of mismatch repair, 78.3%, is similar to that observed previously in yeast for the A:G and C:T mispairs (71–81% BISHOP *et al.* 1989; 65–94% KRAMER *et al.* 1989a) and substantiates our estimate of the average length of heteroduplex DNA.

We next examined several genes in the *RAD52* epistasis group for their role during direct repeat recombination. Previously, it was shown that RAD50 function is not required for mitotic direct repeat recombination (WAGSTAFF *et al.* 1986; GOTTLIEB *et al.* 1989). Similarly, our assay in a *rad50* mutant background is unaffected. Mutations in *RAD52* result in a 2–10-fold reduction in direct repeat recombination in several assays including the assay reported here (2-fold; Table 3) (KLEIN 1988; SCHIESTL and PRAKASH 1988; THOMAS and ROTHSTEIN 1989b). Unlike *rad50* and *rad52* strains, mutations in *RAD51*, *RAD54*, *RAD55* and *RAD57* have not been extensively characterized for their effects on direct repeat recombination. We found that *rad51*, *rad54*, *rad55* and *rad57* mutant strains display increased recombination between direct repeats. This was surprising since mutations in these genes were previously shown to exhibit reduced mitotic heteroallelic recombination (SAEKI *et al.* 1980). One possible way that a hypo-rec mutation for heteroalleles can cause an increase in direct repeat recombination is for the intermediates, in the absence of the Rec function, to be processed via different pathways. Further studies have shown that the hyper-rec phenotype of these *rad* mutants is dependent both on *RAD1* and *RAD52* (J. MCDONALD and R. ROTHSTEIN, manuscript in preparation).

Our results show that the *RAD52* group is comprised of three classes with respect to direct repeat recombination frequency: wild-type level (*rad50*), hypo-rec (*rad52*) and hyper-rec (*rad51*, *rad54*, *rad55* and *rad57*). However, only *rad52* mutant strains exhibit a significant change in the percentage of sectored colonies (nearly 60% increase; Table 4) indicating that there is more unrepaired heteroduplex than in wild-type strains. This increase may be due to either longer heteroduplex tracts or reduced hDNA repair. We favor the former explanation since earlier studies suggest that the length of conversion tracts is longer in *RAD52*-independent recombination (HOEKSTRA *et al.* 1986; DORNFELD and LIVINGSTON 1992).

It has been proposed that *RAD1* may be involved in a *RAD52*-independent pathway for direct repeat recombination (KLEIN 1988; SCHIESTL and PRAKASH 1988; THOMAS and ROTHSTEIN 1989b). Although the frequency of recombination between the *SUP4* repeats is not altered in *rad1* mutants, the percent of sectored colonies is slightly reduced ($P \leq 0.01$). Our result is consistent with the finding of AGUILERA and KLEIN (1989) that conversion tract length is reduced in *rad1* strains. We propose that hDNA tracts are shorter in *rad1* mutant strains and that *RAD1* is involved in the extension and processing of heteroduplex DNA. Recently, FRIEDBERG's laboratory has shown that a Rad1 Rad10 protein com-

plex exhibits single-strand DNA endonuclease activity (TOMKINSON *et al.* 1993; SUNG *et al.* 1993). Perhaps the Rad1 Rad10 endonuclease activity promotes the formation of long heteroduplexes and in its absence shorter hDNA results.

We next examined recombination and sectored colony formation in *rad1 rad52* double mutant strains. Both recombination and percent sectored colonies are synergistically reduced in the double mutant (Table 4). The synergy has been interpreted as evidence that *RAD1* and *RAD52* function in alternate pathways (KLEIN 1988; SCHIESTL and PRAKASH 1988; THOMAS and ROTHSTEIN 1989b). The residual recombination indicates that there must be an additional pathway(s) for direct repeat recombination. Two possible explanations for the reduction in the percent of sectored colonies in *rad1 rad52* double mutant strains are: firstly, the mismatch within heteroduplex is more efficiently corrected and secondly, the length of heteroduplex is reduced. The results in Table 4 support the second hypothesis since the percent sectored colonies increases in *rad1 rad52* double mutants after shortening the repeats. Since a 4.2-fold reduction in percent sectored colonies is found in *rad1 rad52* mutants carrying the 2.3-kb repeats compared to wild-type, we estimate the length of heteroduplex tracts in the double mutant to be approximately 250 bp (1045 bp/4.2-fold). Therefore, it is likely that the *RAD1 RAD52* alternate recombination pathway(s) is characterized by shorter heteroduplex tract length.

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