Genetic Control of Intrachromosomal Recombination in *Saccharomyces cerevisiae.* I. Isolation and Genetic Characterization of Hyper-Recombination Mutations

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ABSTRACT

Eight complementation groups have been defined for recessive mutations conferring an increased mitotic intrachromosomal recombination phenotype (*hpr* genes) in *Saccharomyces cerevisiae.* Some of the mutations preferentially increase intrachromosomal gene conversion (*hpr4*, *hpr5* and *hpr8*) between repeated sequences, some increase loss of a marker between duplicated genes (*hpr1* and *hpr6*), and some increase both types of events (*hpr2*, *hpr3* and *hpr7*). New alleles of the *CDC2* and *CDC17* genes were recovered among these mutants. The mutants were also characterized for sensitivity to DNA damaging agents and for mutator activity. Among the more interesting mutants are *hpr5*, which shows a biased gene conversion in a *leu2-112::URA3::leu2-k* duplication; and *hpr1*, which has a much weaker effect on interchromosomal mitotic recombination than on intrachromosomal mitotic recombination. These analyses suggest that gene conversion and reciprocal exchange can be separated mutationally. Further studies are required to show whether different recombination pathways or different outcomes of the same recombination pathway are controlled by the genes identified in this study.

HOMOLOGOUS recombination is the result of an exchange of information between homologous DNA regions. Recombination may be reciprocal, resulting in crossovers, or nonreciprocal, resulting in gene conversion events. Although conventional recombination studies in yeast use markers on homologous chromosomes (FOGEL, MORTIMER and LUSNAK 1981), recombination also occurs between sequences repeated on the same chromosome (JACKSON and FINK 1981; KLEIN and PETES 1981), and on nonhomologous chromosomes (SCHERER and DAVIS 1980; ERNST, STEWART and SHERMAN 1981; MIKUS and PETES 1982; JINKS-ROBERTSON and PETES 1985, 1986; LICHTEN, BORTS and HABER 1987), in both meiosis and mitosis. Similar mitotic events occur in cultured mammalian cells (SLIGHTOM, BLECHL and SMITHIES 1980; VARMUS, QUINTELL and ORTIZ 1981; ROBERTS, BUCK and AXEL 1983; LISKAY and STACHELEK 1983).

There are several reasons to believe that intrachromosomal and interchromosomal exchanges are mechanistically similar. They require common functions (WAGSTAFF *et al.* 1985) and the DNA repair mutation *rad52-1* (RESNICK 1969), which abolishes homologous recombination (MALONE and ESPOSITO 1980), also reduces intrachromosomal exchanges (JACKSON and FINK 1981; WILLIS and KLEIN 1987).

In mitotic and meiotic recombination events in yeast, gene conversion is associated with reciprocal exchange, although the percent association varies over a wide range (HURST, FOGEL and MORTIMER 1972; HURST and FOGEL 1964; for reviews see ESPOSITO and WAGSTAFF 1981 and ORR-WEAVER and SZOSTAK 1985). Because of this association gene conversion has been made the central event in all models of the recombination process (HOLLIDAY 1964; MESELSON and RADDING 1975; SZOSTAK *et al.* 1983).

It has recently been reported that events selected as intrachromosomal (WILLIS and KLEIN 1987) and interchromosomal (BORTS and HABER 1987) reciprocal exchanges are associated with gene conversion. Nonetheless, mitotic gene conversion can sometimes be dissociated from reciprocal exchange. ROMAN (1956) reported that UV irradiation preferentially increases reciprocal exchange in mitotically growing cells, while ethylmethanesulfonate (EMS) and nitrosoguanidine preferentially increase gene conversion. It has been suggested that mitotic gene conversion can take place in the G_1 and G_2 stages of the cell cycle, while reciprocal exchange is restricted to G_2 (ROMAN and FABRE 1983). In addition, a *rad18* mutation increases mitotic gene conversion but not reciprocal exchange between homologous chromosomes (BORAM and ROMAN 1976).

Most intrachromosomal gene conversion events are not associated with reciprocal exchange either in mitosis (JACKSON and FINK 1981) or in meiosis (KLEIN

and PETES 1981; KLEIN 1984; JACKSON and FINK 1985), although when reciprocal exchanges occur they are associated with gene conversion (WILLIS and KLEIN 1987). Similar results have been obtained with cultured mammalian cells (LISKAY and STACHELEK 1983; LIN, SPERLE and STERNBERG 1984). This has led to the speculation that gene conversion is a mechanism for the maintenance of sequence homogeneity between repeated genes. Suppression of associated reciprocal exchanges would prevent genetic rearrangements (EGEL 1981; BALTIMORE 1981).

Mutations that specifically increase reciprocal exchange or gene conversion would be extremely valuable in understanding the separate but linked recombination events of gene conversion and reciprocal exchange. The first hyper-recombination mutations reported in *Escherichia coli* and *Saccharomyces cerevisiae* were in genes already known to be involved in DNA synthesis or repair. Examples of this in *E. coli* are mutations in the helicase II gene (ARTHUR and LLOYD 1980), in the DNA adenosine methylase (MARINUS and KONRAD 1976; ZIEG, MAPLES and KUSHNER 1978) and in the DNA polymerase I and ligase genes (ZIEG, MAPLES and KUSHNER 1978). Although less well defined, yeast replication and DNA repair functions show a similar overlap (KUNZ and HAYNES 1981). The DNA repair mutations *rad24-1* (r_1s) (KOWALSKY and LAKOWSKY 1975; ECKARDT-SCHUPP, SIEDE and GAME 1987), *rem1* alleles of *rad3* (MONTELONE, HOEKSTRA and MALONE 1988), *rad6* (KERN and ZIMMERMANN 1978), *rad18* (BORAM and ROMAN 1976) and mutations in genes involved in DNA synthesis such as the DNA ligase gene *CDC9* (GAME, JOHNSTON and VON BORSTEL 1979), and the thymidylate kinase gene *CDC8* (HARTWELL and SMITH 1985) have hyper-recombination activity. Characterization of mutations isolated on the basis of a hyper-recombination phenotype in *E. coli* (KONRAD 1977; FEINSTEIN and LOW 1986) and in yeast (MALONEY and FOGEL 1979; ESPOSITO *et al.* 1984; for a review see ORR-WEAVER and SZOSTAK 1985) provide further evidence of the complex relationship between DNA replication, repair and recombination.

To begin a study of the molecular events of intrachromosomal recombination, we have isolated mutants on the basis of increased recombination between repeated sequences. Yeast strains carrying both a *leu2* heteroallelic gene duplication and a *his3* heteroallelic gene duplication were mutagenized and screened by inspection to identify colonies yielding an increased frequency of Leu^+His^+ papillae when grown on selective medium. This approach allowed us to identify hyper-recombination mutants in haploid strains, without imposing a selection for DNA repair or replication defects. Further, the differential effect of the mutations on gene conversion and "pop-out" recombination (loss of a marker between duplicated genes) could be determined separately using the same gene duplication system. We believe we have defined genes that will be important in the understanding of the biochemistry of recombination and the association between gene conversion and reciprocal exchange in intrachromosomal recombination.

MATERIALS AND METHODS

Strains: Most strains used in this study are described in Table 1. In addition, the cell cycle mutants *cdc2*, *cdc5*, *cdc6*, *cdc8*, *cdc9*, *cdc13*, *cdc14*, *cdc15* and *cdc17*, were kindly provided by C. NEWLON and L. HARTWELL. Mutations *cdc5*, *cdc9* and *cdc13* were introduced into our strains from strains H5C1B1, H9C1A1 and H13C1A1 (obtained from L. HARTWELL), respectively. Mutation *cdc14* was obtained from strain C214 (C. NEWLON).

Media and growth conditions: Rich medium YEPD, synthetic media SD, and synthetic complete medium with bases and amino acids omitted as specified, as well as sporulation medium, were prepared as described (SHERMAN, FINK and HICKS 1986). Cells were grown either on solid medium or in liquid medium with rotational shaking at 30°, unless otherwise specified. L-Canavanine sulfate and 5-fluoro-orotic acid (FOA) were added to synthetic medium at concentrations of 60 μg/ml and 750 μg/ml, respectively.

Mutagenesis and mutant screen: Yeast strains were grown overnight in 5 ml YEPD. Cells in early stationary phase were resuspended in 0.1 M sodium phosphate buffer (pH 7.0) to a density of approximately 5×10^7 cell/ml. An aliquot of 30–50 μl of ethylmethane sulfonate (Eastman Kodak Co., Rochester, NY) was added to 1.7 ml of the cell suspension. After 1 hr at 30°, the suspension was added to 8 ml of 5% sodium thiosulfate and washed three times with buffer. Mutations were allowed to segregate by growing the mutagenized cells in 5 ml YEPD at 26° for 6–8 hr. Mutagenized colonies were recovered on YEPD plates after 3 days at 26°. Each colony was either directly replica-plated onto SD-his, SD-leu and SD-his-leu, or transferred to new YEPD plates first and then replica-plated on the three selective media after 2 days. Those colonies showing high frequency of papillation over the wild type were selected as putative hyper-recombination mutants.

UV and MMS sensitivity: Methylmethanesulfonate (MMS) is a chemical alkylating agent that leads to single-stranded DNA breaks (PRAKASH and STRAUSS 1970). MMS sensitivity was determined by plating serial dilutions of overnight YEPD cultures onto freshly prepared YEPD plates supplemented with 0.02% MMS (Eastman Kodak Co., Rochester, NY). Colonies were counted after 3 days at 30°. To determine UV sensitivity, the yeast cells were grown overnight in 5 ml of SD complete medium, plated on SD complete medium and irradiated with a 40 W UV lamp from 30 to 120 J/m^2. Plates were covered with aluminum foil, and colonies were counted after 3 days at 30°. In both types of experiments total cell number was obtained by plating appropriate dilutions of the same cultures used in the MMS and UV experiments onto YEPD and SD complete medium. Colonies were counted after 2 days at 30°.

Genetic analysis of the hyper-recombination mutations: Genetic analysis was performed as described (SHERMAN, FINK and HICKS 1986). The hyper-recombination phenotype could not be visualized unambiguously by replica-plating onto selective media, so a "qualitative mini-fluctuation test" was performed with each haploid or diploid strain.

TABLE 1

Strains

344-8D	*MAT***a** *leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52*
344-109D	*MAT***a** *leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 ade1-101*
344-27C	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 ade1-101*
344-115B	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1*
305-12D	*MATα leu2-101::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1*
MA-4B	*MAT***a** *leu2-k::URA3::leu2-r his3-513::TRP1::his3-537 trp1 ura3-52*
X260-2B	*MATα ura3-52 rad52-1*
X260-3A	*MAT***a** *ura3-52 rad52-1*
8D8-3A	*MAT***a** *leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 ade1-101*
109D26-6A	*MATα leu2-112::URA3::leu2-k HIS3::TRP1::his3 ura3-52 trp1 ade1-101*
H5-1A	*MAT***a** *leu2-112::URA3::leu2-k trp1 ura3-52 cdc5*
H9-2-15A	*MAT***a** *leu2-112::URA3::leu2-k his3-513::TRP1::his-537 ura3-52 cdc9*
H13-2-15A	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 ade1-101 cdc13*
214-7A	*MATα leu2-112::URA3::leu2-k ade2 cdc14*
H19-3	*MATα ade2-1 can1-100 his3-11,15 ura3-1 trp1-1 leu2-3,112*
8D8-3B	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 ade1-101 hpr1*
8D8-4B	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 ade1-101 hpr1*
8D20-7D	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 hpr2*
109D26-6C	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 ade1-101 hpr3*
109D26-6D	*MAT***a** *leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 ade1-101 hpr3*
27C6-8A	*MAT***a** *LEU2::URA3::leu2 his3-513::TRP1::his3-537 trp1 ura3-52 hpr4*
27C6-8D	*MATα leu2-112::URA3::leu2-k HIS3::TRP1::his3 ura3-52 ade1-101 hpr4*
27C14-3D	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 hpr5*
27C14-4A	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 ade1-101 hpr5*
27C14-4B	*MAT***a** *leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 hpr5*
27C22-1C	*MAT***a** *leu2-112::URA3::leu2-k ura3-52 ade1-101 hpr6*
27C22-3C	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 ade1-101 trp1 hpr6*
12D53-4B	*MAT***a** *leu2-r::URA3::leu2-k his3-513::TRP1::his3-537 trp1 ura3-52 hpr7*
12D53-5D	*MAT***a** *leu2-r::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 hpr7*
12D78-2D	*MATα leu2-r::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 ade1-101 hpr8*
12D78-5C	*MAT***a** *leu2-k::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 trp1 hpr8*
14-260-2D	*MATα leu2-112::URA3::leu2-k ura3-52 trp1 ade1-101*
14-260-4B	*MATα leu2-112::URA3::leu2-k ura3-52 trp1 rad52-1*
14-260-4D	*MATα leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 ade1-101 hpr5 rad52-1*
8-260-5C	*MATα leu2-112::URA3::leu2-k trp1 ura3-52 hpr1 rad52-1*
8-260-10B	*MATα leu2-112::URA3::leu2-k trp1 ura3-52 hpr1 rad52-1*
26-260-11A	*MATα leu2-112::URA3::leu2-k trp1 ura3-52 hpr3 rad52-1*
26-260-17B	*MATα leu2-112::URA3::leu2-k trp1 ura3-52 hpr3 rad52-1*
8D8AC57	*MAT***a** *ade1-101 leu2-112 his3-513::TRP1::his3-537 trp1 ura3-52* *MATα ADE1 leu2-k HIS3::TRP1::his3 TRP1 ura3-52*
8D8BD43	*MAT***a** *ade1-101 leu2-112 his3-513::TRP1::his3-537 trp1 ura3-52 hpr1* *MATα ADE1 leu2-k HIS3::TRP1::his3 TRP1 ura3-52 hpr1*
8BD48-4-8A	*MAT***a** *ade1-101 leu2-112::URA3::leu2-k trp1 ura3-52 can1-100 hpr1* *MATα ade1-101 LEU2 trp1 ura3-52 CAN1 hpr1*
109D26CD34	*MAT***a** *ade1-101 leu2-112 his3-513::TRP1::his3-537 trp1 ura3-52 hpr3* *MATα ade1-101 leu2-k HIS3::TRP1::his3 trp1 ura3-52 hpr3*
27C6BD26	*MAT***a** *ade1-101 leu2-112 HIS3 trp1 ura3-52 hpr4* *MATα ADE1 leu2-k his3 trp1 ura3-52 hpr4*
27C14BB53	*MAT***a** *ade1-101 leu2-112 HIS3 trp1 ura3-52 hpr5* *MATα ADE1 leu2-k his3 trp1 ura3-52 hpr5*
27C14BA84	*MAT***a** *leu2-112::URA3::leu2-k his3-513::TRP1::his3-537 ura3-52 ade1-101 can1-100 hpr5* *MATα LEU2 HIS3 ura3-52 ADE1 CAN1 hpr5*
12D53B37	*MAT***a** *ade1-101 leu2-112 HIS3 trp1 ura3-52 hpr7* *MATα ADE1 leu2-k his3 trp1 ura3-52 hpr7*

All strains listed have been constructed for this study. The exception is H19-3, a derivative of strain H19 from R. ROTHSTEIN's laboratory. The origin of some of the markers used is indicated in the text.

This "mini-test" was done on four colonies for each strain. A colony was resuspended in 1 ml water and an aliquot of 25 μl of this suspension was plated onto the selective media SD-leu and SD-his. Frequency of papillation was used to score the hyper-recombination phenotype. The monogenic nature of each mutation was established by analysis of four to five complete tetrads. Complementation tests were performed by direct analysis of the recombination frequency of the doubly heterozygous diploids, since all eight mutations were recessive. These heterozygous diploids were constructed with strains from the first outcross, which allowed recovery of each mutant in both *MAT* backgrounds. Linkage

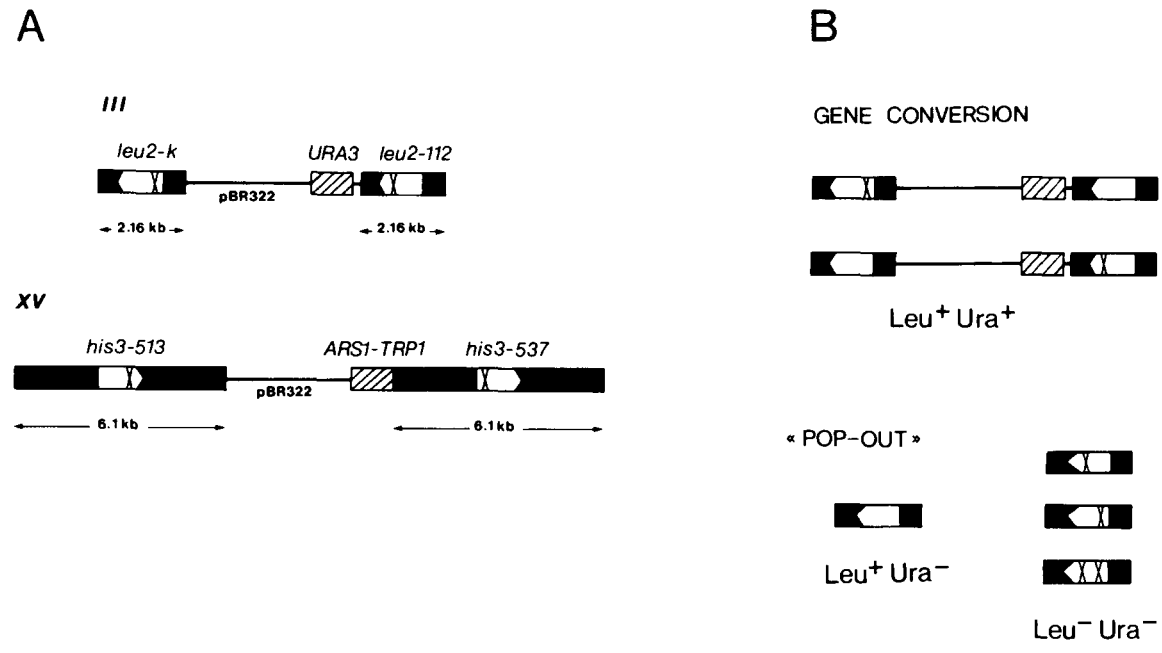

FIGURE 1.—A, Duplications used to study intrachromosomal recombination. B, Selectable genetic products of gene conversion and "pop-out" for the *leu2* duplication.

of mutations *hpr1*, *hpr2*, *hpr3*, *hpr4*, *hpr5* and *hpr6* was studied by tetrad analysis. The recovery of spores without a hyper-recombination phenotype confirmed that each mutation mapped to a separate locus.

Analysis of recombination rates: Recombination rates were calculated according to the fluctuation test of LEA and COULSON (1948). Strains were grown on solid YEPD medium at 30° for 2 or 3 days, and up to 18 colonies from each strain were used for each fluctuation test performed with each different strain. None of the mutations affected viability, so cell number was determined in a hemocytometer. To determine the recombination rate between homologous chromosomes at the *CENIII-MAT* region, aliquots from eight independent cultures, from each strain tested, were separately mixed in water with a 10-fold excess of cells of each mating tester strain. The mixed cultures were plated on YEPD plates and allowed to mate for 7 hr before being replica-plated onto selective minimal medium. The mutator phenotype was determined as the rate of mutation of the allele *CAN1*^{s} to *can1*^{r}.

DNA manipulation: Plasmid DNA was isolated from *E. coli* by CsCl gradient centrifugation as described (CLEWELL and HELINSKI, 1970). Yeast genomic DNA was prepared from 5 ml YEPD cultures according to SHERMAN, FINK and HICKS (1986). After digestion with the specified restriction endonucleases, chromosomal DNA fragments were fractionated by agarose gel electrophoresis. DNA was transferred to nitrocellulose filters according to SOUTHERN (1975). Yeast colony hybridization was performed as described by SHERMAN, FINK and HICKS (1986). ^{32}P-labeled DNA plasmids were prepared according to FEINBERG and VOGELSTEIN (1984). Hybridization was performed in 6× SSC, 1× Denhardt's solution, 0.25 M NaH_2PO_4 pH 6 and 30 mM tetrasodium pyrophosphate at 65° for 18 hr. X-OMAT Kodak film was used for autoradiography.

RESULTS

Isolation of single gene mutations with a hyper-recombination phenotype: *Monitoring intrachromosomal recombination.* Two chromosomal duplications were used to detect intrachromosomal recombination (Figure 1). The first is a duplication of a 2.16-kb *Sal*I-*Xho*I DNA fragment containing the *LEU2* gene, on the left arm of chromosome *III* at the *LEU2* locus. One copy of this sequence carries the allele *leu2-k*, a deletion of 7 bp at the *Kpn*I site in the *LEU2* coding region (H. KLEIN, unpublished results). The other copy carries the ICR170 induced mutation allele *leu2-112*, which is thought to be a +1 frameshift mutation (ERNST, HAMPSEY and SHERMAN 1985; MATHISON and CULBERTSON 1985; M. CULBERTSON, personal communication). The plasmid pBR322 carrying the yeast *URA3* gene at the unique *Hin*dIII site is located between the *leu2* copies. The second marker system is a duplication of a 6.1-kb *Eco*RI-*Sal*I DNA fragment containing the *HIS3* gene on the right arm of chromosome *XV*, at the *HIS3* locus. One copy of this sequence carries the allele *his3-513*, which is a loss of the *Kpn*I site in the *HIS3* coding region, and the other copy carries the allele *his3-537*, which is a loss of the *Hin*dIII site distal to the *Kpn*I site (T. ORR-WEAVER, unpublished results). The plasmid pBR322 carrying the yeast *ARS1-TRP1* sequence at the unique *Eco*RI site is located between the *HIS3* copies.

One of the original strains (305-12D) used to screen for hyper-recombination mutants carried a different *leu2* duplication. This was *leu2-101::URA3::leu2-112*. The *leu2-101* allele (from G. FINK) was used in place of the *leu2-k* allele. For other studies the *leu2-112* allele was substituted by the *leu2-r* allele, which is a loss of the *Eco*RI site (BORTS, LICHTEN and HABER 1986), to give the duplication *leu2-r::URA3::leu2-k*.

Strains carrying these duplications and the *trp1* and the *ura3-52* alleles at the original *TRP1* and *URA3* loci were used to monitor intrachromosomal recombination. Retention of the duplication with one wild-type allele and one of the original alleles was scored as gene conversion (JACKSON and FINK 1981). Leu^+Ura^+ or His^+Trp^+ recombinants were both scored as gene conversion events. Southern analysis of this class of recombinants confirmed the expected pattern of bands for gene conversion events (Figure 1). In the *leu2* duplication, loss of the *URA3* gene (Ura^-) is called a pop-out event (RONNE and ROTHSTEIN 1988), whether or not the recombinant is Leu^+ (Figure 1). We confirmed by colony hybridization that the Ura^- recombinants have lost the pBR322 sequences. Leu^+Ura^- recombinants are called Leu^+ pop-out events. They represent either the proportion of Leu^+ segregants in a population of selected Ura^- recombinants or the proportion of Ura^- segregants in a population of selected Leu^+ recombinants. In classifying events at the *his3* duplication, the His^+Trp^- recombinant class is the equivalent to the Leu^+Ura^- class (His^+ pop-out).

There are three ways to explain a pop-out of the *URA3* gene from the *leu2* duplication or of the *TRP1* gene from the *his3* duplication: by intrachromosomal reciprocal exchange at the G_1 or G_2 stage of the cell cycle; by unequal sister chromatid exchange at G_2; and by gene conversion in G_2 between one whole *leu2* duplication from one chromatid and one of the *leu2* alleles in the sister chromatid (ROTHSTEIN, HELMS and ROSENBERG 1987). We cannot distinguish between these three possibilities with the duplications used in this study.

Isolation of hyper-recombination mutations. Strains 344-8D, 344-27C, 344-109D, 344-115B and 305-12D (Table 1), each carrying a *leu2* and *his3* duplication, were mutagenized with EMS to a viability ranging from 10 to 40%. From the first four strains (344-8D, 344-27C, 344-109D, 344-115B) a total of 21,380 mutagenized colonies were transferred with toothpicks from the original plates to new YEPD plates and replica-plated after two days onto the selective media SD-his, SD-leu and SD-his-leu. From strain 305-12D 500 plates each containing approximately 100 mutagenized colonies were directly replica-plated onto SD-his-leu medium.

All putative mutants surviving three rounds of screening for hyper-recombination by papillation on SD-his, SD-leu and SD-his-leu media were subjected to mini-fluctuation tests (see MATERIALS AND METHODS). A total of 21 putative hyper-recombination mutants were chosen. Recombination frequencies were calculated for the mutant strains and compared to the wild-type values. Mutagenized strains with an increased recombination frequency leading to Leu^+His^+, Leu^+ or His^+ recombinants of at least 10-fold over the wild-type level were selected for further genetic studies. Genetic analysis of the 16 selected strains showed that 8 mutations clearly segregated 2:2 for the hyper-recombination phenotype. Each mutation was recessive and defined a different complementation group, as determined by tetrad analysis of double heterozygous mutant crosses, or by direct analysis of the recombination frequency of the diploids. The remaining mutants did not show a clear monogenic segregation, possibly because the mutant phenotype was weak and hence difficult to score. One of these also showed a very poor mating ability, and another was UV sensitive. These mutants were excluded from further analysis.

Spore segregants carrying the *leu2-112::URA3::leu 2-k* and *his3-513::TRP1::his3-537* duplications and one hyper-recombination mutation, *hpr*, were selected for further study. Recombination rates for each duplication were determined using several spores for each mutation. As wild-type strains, we used strains derived from the same crosses from which the mutant strains were obtained. These wild-type strains did not show major differences in recombination rate compared to the parental wild-type strains (344-8D, 344-27C, 344-109D and 344-115B). Table 2 shows that *hpr1*, *hpr3*, *hpr6* and *hpr7* are strong hyper-recombination mutations. The increase in the rate of His^+ or Leu^+ recombinants ranges from 14- to 100-fold, and the increase in Ura^- recombinants ranges from 20- to 400-fold. The remaining mutants, *hpr2*, *hpr4*, *hpr5* and *hpr8*, have recombination rates of His^+ or Leu^+ segregants that are increased approximately 10-fold over the wild-type levels. The rate of appearance of Ura^- recombinants in this latter group of mutants was variable, but was never more than 10-fold increased over the wild-type value. *hpr1* and *hpr6* showed greater rates of Ura^- segregants than Leu^+ or His^+ recombinants. The reverse was true for other mutants such as *hpr4* and *hpr5*.

Relationship between gene conversion and pop-out recombination: To establish whether a mutation preferentially enhanced one type of event, as suggested by the analysis of Ura^- pop-outs above, independent Leu^+ or His^+ recombinants were selected from each *hpr* strain. These were scored for uracil and tryptophan prototrophy (Table 3). In the wild-type strain 45% of recombinants selected as Leu^+ were also Ura^+, and 50% of recombinants selected as His^+ were also Trp^+.

The fraction of gene convertants among the Leu^+ recombinants (Leu^+Ura^+ recombinants) fell to 10% in the mutants *hpr1* and *hpr6*. Colony hybridization experiments were performed with 50 independent Ura^- recombinants for each mutant, to confirm that all Ura^- segregants had lost the pBR322 sequence lo-

TABLE 2

Rates of mitotic intrachromosomal recombination

Strains	IC[a]	Rate (× 10^6) His$^+$	Leu$^+$	Ura$^-$
Wild type				
8D8-3A	18	2.8	6.2	8.2
27C22-1B	18	5.6		
109D	12	4.9	9.7	22
109D26-6A	10		22	
305-12D	12	5.3		
MA-4B	12	3.9	6.5	39
Wild type average		4.5	11.1	23
Mutants				
hpr1	36	88–130 (×24)[b]	140–260 (×18)	850–1200 (×45)
hpr2	22	156 (×35)	40–68 (×5)	89 (×4)
hpr3	22	200–300 (×56)	510–520 (×46)	1100–1140 (×49)
hpr4	22	58 (×13)	91–210 (×14)	71–250 (×7)
hpr5	64	34–58 (×10)	45–140 (×8)	15–32 (×1)
hpr6	26	96 (×21)	490–1850 (×105)	9500 (×413)
hpr7	24	120 (×27)	140–170 (×14)	450 (×20)
hpr8	24	37 (×8)	43 (×4)	83–130 (×9)

One fluctuation test was performed for each wild-type strain. One to three tests were performed for each mutant: strains 8D8-3B and 8D8-4B (*hpr1*); 8D20-7D (*hpr2*); 109D26-6C and 109D26-6D (*hpr3*); 27C6-8A and 27C6-8D (*hpr4*); 27C14-3D, 27C14-4A and 27C14-4B (*hpr5*); 27C22-1C and 27C22-3C (*hpr6*); 12D53-5C and 12D53-5D (*hpr7*); 12D78-2D and 12D78-5C (*hpr8*). The rate calculated for each test is given as recombinants/cell/generation.

[a] IC indicates the total number of independent clones examined.

[b] The number in parentheses is the average increase over the wild-type average rate of median values.

cated between the *leu2* duplication. The fraction of Trp$^+$ segregants among the His$^+$ recombinants was also reduced, although not as drastically. Together with the higher rates of Ura$^-$ recombinants than Leu$^+$ recombinants found for *hpr1* and *hpr6* (Table 2), these results suggest that mutations *hpr1* and *hpr6* preferentially increase a pop-out type of recombination event. Conversely, the mutations *hpr4*, *hpr5* and *hpr8* produced Leu$^+$ recombinants by gene conversion (Leu$^+$Ura$^+$ recombinants). About 70–90% of Leu$^+$ recombinants in these three mutants were gene convertants (Leu$^+$Ura$^+$). This was confirmed by Southern blots for about 70 independent gene convertants in *hpr4* and *hpr5* mutants. Similar results were obtained with the *his3* duplication, although the differences were not as striking. A varied but similar increase for both types of recombination events was observed in the remaining mutants (Table 3). For these mutants, the proportion of pop-out events in the *leu2-112::URA3::leu2-k* interval leading to Leu$^+$Ura$^-$ recombinants is not significantly different from wild type.

Studies on DNA repair: Many enzymes involved in DNA repair are also involved in recombination or replication. A number of mutants that show repair deficiencies also have a hyper-recombination phenotype (HAYNES and KUNZ 1981). We therefore characterized the ability of strains bearing *hpr* mutations to repair spontaneous mutations, UV and MMS induced DNA damage (Table 4). Spontaneous mutation levels (mutator activity) were determined by the rate of mutation of the *CAN1* wild-type allele to *can1^r* alleles spontaneously. UV and MMS sensitivity for growth were used to estimate the ability of the mutants to repair UV and MMS induced DNA damage (see MATERIALS AND METHODS). *hpr6* increased mutation at the *CAN1* locus 50-fold over wild type. Mutations *hpr3* and *hpr4* were weaker mutators (5–8-fold increases). Only *hpr5* affected the repair of UV induced DNA damage. *hpr3* and *hpr6* mutants were defective in repair of DNA breaks induced by MMS. *hpr5* mutants were slightly MMS sensitive. Thus, four of the hyper-recombination mutants (*hpr1*, *hpr2*, *hpr7* and *hpr8*) showed no phenotype related to DNA repair functions.

***hpr3* and *hpr6* are mutations in *CDC* genes:** The mutants *hpr3* and *hpr6* failed to grow at 37°. This temperature sensitivity co-segregated with the hyper-recombination phenotype. Nine of the *cdc* mutants show increased mitotic recombination between homologous chromosomes (HARTWELL and SMITH 1985), so we tested these for allelism to *hpr3* and *hpr6*. *hpr3* was allelic to *cdc17* and *hpr6* was allelic to *cdc2*. We observed that cells carrying either a *hpr3* or *hpr6* mutation arrested growth as doublets when shifted to 37°, as did the original *cdc2* and *cdc17* alleles (PRINGLE and HARTWELL 1981).

CDC2 is required for DNA synthesis. Mutant *cdc2* cells fail to replicate about one third of the total DNA (CONRAD and NEWLON 1983) at 37°. *CDC17* is the

TABLE 3

Relationship between intrachromosomal gene conversion and reciprocal exchange in mitosis

	Independent experiments[a]	Ura⁻		Leu⁺		His⁺	
		Leu⁺:Leu⁻	% Leu⁺ [b]	Ura⁺:Ura⁻	% GC[c]	Trp⁺:Trp⁻	% GC
Wild type	All	23:131	14.9	116:147	44.1	94:95	49.7
	64	119:691	14.7	389:470	45.3	49:40	55.1
hpr1	All	5:72	6.5	12:150*	7.4	30:75*	28.6
	40	148:622	19.0	112:784	12.5	117:403	22.5
hpr2	All	53:191*	21.7	52:128*	28.9	ND[d]	
	8			57:94	37.7		
hpr3	All	8:99*	7.5	30:36	45.4	ND	
	14			115:144	44.4	99:78	55.6
hpr4	All	21:124	16.8	137:39*	77.8	ND	
	8	16:98	15.4	198:29	87.2		
hpr5	All	33:132	20.0	115:10*	92.0	89:27*	76.7
	58	22:127	14.8	778:96	89.0		
hpr6	All	13:85	13.3	8:67*	10.7	10:19*	34.5
	22			52:483	9.7		
hpr7	All	ND		58:42	58.0	ND	
hpr8	All	ND		134:20*	87.0	87:16*	84.5

Independent Ura⁻, Leu⁺ or His⁺ recombinants were selected and the associated leucine, uracil and tryptophan phenotypes were determined. Wild-type strains used were 344-8D, 344-27C, 344-109D, 344-15B, 8D8-3A and 109D26-6A. Mutant strains used are those given in Table 2.

[a] Two different sets of experiments are reported here: *all* indicates that each recombinant clone analyzed was selected from an independent experiment; a *number* indicates the total number of independent experiments performed from which the recombinant clones were obtained. χ^2 values were calculated for the set of experiments in which each recombinant analyzed came from an independent event. Those in which the difference compared to wild-type values is significant at $P = 0.05$ are indicated with (*).

[b] % Leu⁺: percentage of Ura⁻ recombinants that are Leu⁺.

[c] % GC: percentage Ura⁺ segregants among the Leu⁺ recombinants or percentage of Trp⁺ segregants among the His⁺ recombinants tested.

[d] ND: not determined.

TABLE 4

Mutator phenotype, UV and MMS sensitivity

	Frequency Can^r ($\times 10^6$)[a]	Percent survival	
		UV (70 J/m^2)[b]	MMS (.01%)[c]
Wild type	5	30–50	30–50
hpr1	3	42	23
hpr2	9	36	59
hpr3	26	33	3.5
hpr4	38	33	46
hpr5	3	4.7	10
hpr6	280	40	1.3
hpr7	6	34	38
hpr8	8	51	40

Strains used were 8D8-3A, 109D26-6A and MA-4B (wild-type strains); 8D8-3B and 8D8-4B (*hpr1*); 8D20-7D (*hpr2*); 109D26-6C and 109D26-6D (*hpr3*); 27C6-8A and 27C6-8D (*hpr4*); 27C14-4A and 27C14-4B (*hpr5*); 27C22-1C and 27C22-3C (*hpr6*); 12D53-4B and 12D53-5D (*hpr7*) and 12D78-2D and 12D78-5C (*hpr8*). The entries are the average values obtained with each different strain.

[a] Median frequency of spontaneous canavanine resistant colonies obtained from eight independent experiments for each strain.

[b] Irradiation experiments with 50, 90 and 120 J/m^2, also showed that *hpr5* is the only UV sensitive mutant.

[c] Replica-plating experiments onto YEPD supplemented with 0.01, 0.02 and 0.04% MMS clearly showed similar results.

structural gene for DNA polymerase I in yeast (M. J. CARSON and L. H. HARTWELL, personal communication) and is involved in completing telomere elongation following replication (CARSON and HARTWELL 1985). The enhancement of pop-out events in our two *CDC* alleles, both involved in DNA replication, suggested that mutations that affect DNA replication might increase intrachromosomal pop-out recombination. We therefore examined the effect of four other *cdc* mutants on intrachromosomal recombination (Table 5). Mutation in *CDC9*, the structural gene for DNA ligase (JOHNSTON and NASMYTH, 1978) whose product is required in S phase, resulted in a preferential enhancement of Leu⁺ pop-out events (Leu⁺Ura⁻), similar to the results obtained with the *CDC2* allele *hpr6*. Mutation in the *CDC13* gene, whose product is required in the G_2 phase, resulted in an enhancement of both pop-out (Ura⁻) and gene conversion (Leu⁺Ura⁺) events. This pattern of enhancement is similar to the results obtained with the *CDC17* allele *hpr3*. Mutations in *CDC5* and *CDC14*, which result in a block at the M phase, led to a varied enhancement of gene conversion and pop-out recombination events at semipermissive temperature (Table 5).

TABLE 5

Mitotic intrachromosomal recombination in *cdc* mutants

	Frequency of Leu$^+$ ($\times 10^4$)[a]	Leu$^+$		
		Ura$^+$	Ura$^-$	% GC[b]
Wild type	1.2	116	147	44.1
cdc5	10	241	155	60.9
cdc9	29	65	301	17.8
cdc13	8.5	241	282	46.1
cdc14	5.1	49	221	18.1

Strains used were those listed in Table 3 for the wild-type values and the following *cdc* mutant strains: H5-1A (*cdc5*), H9-2-15A (*cdc9*), H13-2-15A (*cdc13*) and 214-7A (*cdc14*).

[a] Each value is the average value from four different colonies grown at 26° overnight, then shifted for 4 hr to 37° and plated on selective medium.

[b] Percentage of Leu$^+$Ura$^+$ segregants among the total Leu$^+$ recombinants tested.

TABLE 6

Effect of the *rad52-1* mutation on hyper-recombination mutants

	Rate ($\times 10^6$)	
	Leu$^+$	Ura$^-$
HPR RAD52[a]	11.1	23
HPR rad52-1	2.0–3.0	7.4
hpr1 rad52-1	1.5–2.0	8–11
hpr3 rad52-1	1.6	4.6
hpr5 rad52-1	1.5–2.0	2.5–6.0

Rates of recombination (recombinant/cell/generation) leading to Leu$^+$ and Ura$^-$ were determined in the duplication *leu2-112::URA3::leu2-k*. Strains used were 14-260-4B (*HPR rad52-1*), 8-260-5C and 8-260-10B (*hpr1*), 26-260-11A and 26-260-17B (*hpr3*), and 14-260-4D (*hpr5*).

[a] Data for the wild-type strain (*HPR RAD52*) are the average values from the median rates taken from Table 1.

The *rad52-1* mutation abolishes hyper-recombination phenotypes: We wanted to test whether the *RAD52* recombinational repair pathway was required for the expression of the hyper-recombination phenotypes. The effect of *hpr1*, which preferentially increases pop-outs, *hpr5*, which preferentially increases gene conversion, and *hpr3*, which increases both types of events, were studied in a *rad52-1* mutation background (Table 6). The *rad52-1* mutation abolished the hyper-recombination phenotype in all cases.

Mitotic recombination between homologous chromosomes: The rate of mitotic recombination between allelic genes situated on homologous chromosomes was examined in several of the mutants (*hpr1*, *hpr3*, *hpr4*, *hpr5* and *hpr7*) (Table 7). Intragenic recombination was studied at the *LEU2* locus using the *leu2-k* and *leu2-112* alleles. Leu$^+$ segregants were scored as recombinants. Intergenic recombination was examined in the *CENV-CAN1* region in heterozygous *CAN1/can1-100* strains. Canavanine-resistant segregants, of genotype *can1-100/can1-100*, were scored as recombinants. Recombination was examined in the *CENIII-MAT* region in *MAT***a**/*MATα* heterozygous

TABLE 7

Rate of allelic recombination between homologous chromosomes in mutant and wild-type strains

	($\times 10^7$) *LEU2*	($\times 10^6$) *MAT***a**	($\times 10^6$) *MATα*	($\times 10^6$) *can1-100*	Sporulation[a]
Wild type	3.5	14	34	6	+
hpr1	10.5	48	75	23	+
hpr3	68	148	467	ND[b]	+
hpr4	35	65	239	ND	+
hpr5	13	75	104	15	+
hpr7	99	35	142	ND	+

Diploid strains used were 8D8AC57 (wild type), 8D8BD43 and 8BD48-4-8A (*hpr1*), 109D26CD34 (*hpr3*), 27C6BD26 (*hpr4*), 27C14BB53 and 27C14BA84 (*hpr5*), and 12D53B37 (*hpr7*).

Alleles shown are those whose phenotype is expressed by the recombinant diploid selected. Rates (recombinant/cell/generation) were calculated from eight independent cultures for each strain used.

[a] Sporulation was determined on KAc medium at 30°

[b] The *HPR3* allele *cdc17* has been shown to increase homologous mitotic recombination at the *CAN1* locus (HARTWELL and SMITH 1985).

strains. *MATα*/*MATα* and MAT**a**/MAT**a** recombinants were identified as colonies able to mate to haploid strains of the opposite mating type (see MATERIALS AND METHODS). Despite its hyper-recombination phenotype when scored for intrachromosomal events, the mutant *hpr1* showed only marginal increases over wild-type rates in these three interchromosomal regions. Mutants *hpr3*, *hpr4*, *hpr5* and *hpr7*, however, showed increases in the rates of mitotic homologous recombination similar to those found for intrachromosomal recombination.

Bias in intrachromosomal gene conversion in *hpr5* strains: *hpr4*, *hpr5*, and *hpr8* mutants preferentially increase the rate of production of Leu$^+$Ura$^+$ recombinants (gene conversion). The rate of production of Leu$^+$Ura$^+$ recombinants was 10-fold higher ($4.8–6.9 \times 10^{-5}$) than the wild-type value (5.1×10^{-6}), as determined from the frequency of recombinants selected directly on SD-leu-ura medium. This 10-fold increase can account for the 10–15-fold increase in the rate of Leu$^+$ or His$^+$ recombinants (Table 2). The rate of production of Leu$^+$Ura$^+$ in the *hpr1* mutant, which preferentially increases pop-outs, was increased only 3–5-fold (1.4×10^{-5}), in contrast to the 50-fold increase in the rate of pop-outs (Table 3).

To search for bias in the direction of gene conversion in these mutants, we examined intrachromosomal gene conversion between the alleles *leu2-k* and *leu2-112* by Southern blot experiments in the mutants *hpr1* and *hpr5*. Figure 2 shows that in the wild-type strain, the allele *leu2-k* was converted to wild type in 57% of the Leu$^+$ cases. In the mutant *hpr1*, which weakly affects the rate of gene conversion in the *leu2* duplication system, the *leu2-k* allele was converted to wild type in 61% of the cases, which is not significantly different from the wild-type value. However, in the

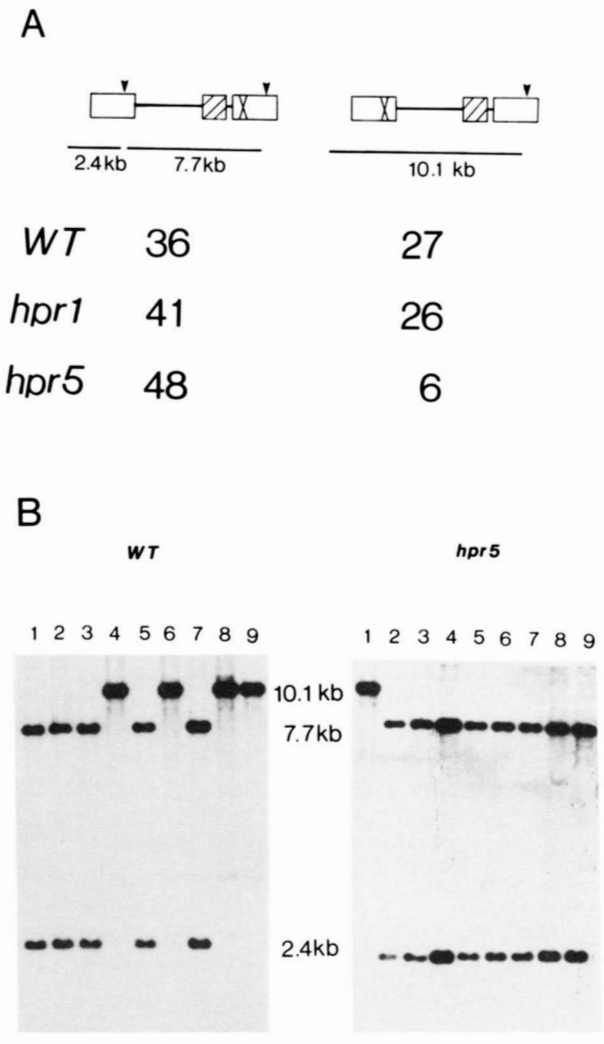

FIGURE 2.—A, Southern blot analysis of independent gene convertants selected in the wild-type strain (*WT*) and the mutants *hpr1* and *hpr5*. The left column shows those segregants which result from a conversion of the *leu2-k* mutation to wild type, and the right side conversions of the *leu2-112* mutation. The arrows represent *Kpn*I sites. B, Southern blots showing the pattern of gene conversion of nine independent recombinants from wild-type and *hpr5* strains. Those lanes showing two hybridizing bands (7.7 and 2.4 kb) correspond to strains which converted the *leu2-k* allele to wild type, and those showing one hybridizing band (10.1 kb) correspond to the recombinants which converted the *leu2-112* allele. Genomic DNA was digested with *Kpn*I. Plasmid pHK135 containing the *Sal*I-*Cla*I *LEU2* fragment in the *Sal*I-*Cla*I site of pBR322, was used as probe.

hpr5 strains studied, the *leu2-k* allele was converted to wild type in 89% of the cases, which is significantly different ($P < 0.05$) from the wild-type value.

DISCUSSION

Mutations have been described that enhance recombination rates in bacteria, yeast and higher eukaryotes (see Introduction). Most of the hyper-recombination mutations that have been isolated also affect DNA repair and/or replication (KONRAD 1977; ZIEG, MAPLES and KUSHNER 1978; KUNZ and HAYNES 1981; ESPOSITO *et al.* 1984; HARTWELL and SMITH 1985; FEINSTEIN and LOW 1986). We have isolated mutations that produce a hyper-recombination phenotype in the yeast *S. cerevisiae*, by screening directly for increased recombination. The eight mutations, which fall into eight complementation groups, vary in the hyper-recombination enhancement. Some are also associated with mutator activity or sensitivity to MMS or UV. The mutations can be put into three classes, based on their effects on intrachromosomal recombination. Mutations in the first class, *hpr1* and *hpr6*, preferentially enhance pop-out recombination. Mutations in the second class, *hpr4*, *hpr5* and *hpr8*, preferentially increase gene conversion events. Mutations in the third class, *hpr2*, *hpr3* and *hpr7*, enhance both types of events.

Two major classes of mutations can be imagined that lead to increased mitotic recombination: (1) those causing deficiencies in DNA repair or replication, thus leading to an accumulation of lesions in the DNA that are recombinational substrates, and (2) those directly or indirectly affecting enzymes involved in recombination. Consistent with previous observations in yeast (HARTWELL and SMITH 1985) and bacteria (ZIEG, MAPLES and KUSHNER 1978), we have recovered mutations that affect DNA replication in our screen for hyper-recombination mutants. Among the eight mutants isolated, we have found two mutants that are defective in genes involved in DNA replication. These were *hpr3*, allelic to *CDC17*, the structural gene for DNA polymerase I (M. J. CARSON and L. H. HARTWELL, personal communication), and *hpr6*, allelic to *CDC2*, required in the initiation process of replication (CONRAD and NEWLON 1983). Two of the mutations did not display any phenotype that could be associated with DNA replication or repair other than the hyper-recombination phenotype itself. These are the mutations *hpr1* and *hpr7*. These two mutations are good candidates for genes with a specific action on recombination.

The mutation *hpr1* produces an approximately 20-fold increase in intrachromosomal recombination, leading to His^+ or Leu^+ segregants. Most of these arise as pop-out events. The increase in the rate of pop-out recombination in the *LEU2* duplication (Ura^-) was 50-fold (Table 2). However, the rate of intrachromosomal gene conversion in this duplication (Leu^+Ura^+) is increased only 3–5-fold. Most of the increased recombination can be explained by a pop-out mechanism. This is consistent with the observation that more than 90% of Leu^+ recombinants were Ura^- (Table 3). As no difference in the pattern of gene conversion of the allele *leu2-k* in the mutant *hpr1* compared to the wild-type strain was seen, this suggests that there are no major alterations in the gene conversion mechanism of the *hpr1* strain (Figure 2).

From the three types of events that can result in pop-out events (ROTHSTEIN, HELMS and ROSENBERG 1987; see first section of RESULTS), we do not believe that pop-out events in the *hpr1* mutants are generated through gene conversion between sister chromatids in G_2, as a general enhancement in gene conversion in the mutant *hpr1* should produce a much larger increase in the rate of production of Leu^+Ura^+ segregants than is observed. Preliminary observations suggest that *hpr1* increases the rate of G418 resistance through reciprocal exchange in an inverted repeat of 360 bp flanking the bacterial Kan^r gene (WILLIS and KLEIN 1987) by 10-fold, suggesting that *hpr1* increases a reciprocal exchange type of event.

The strong effect on intrachromosomal recombination resulting in pop-out events displayed by the *cdc* mutants *hpr3* and *hpr6*, and by the mutations *cdc9* (affected in DNA ligase) and *cdc13* (required during the G_2 stage of the cell cycle) (Tables 3 and 5) suggests that mutations that affect the S phase or G_2 phase of the cell cycle enhance intrachromosomal recombination between repeats. Intrachromosomal recombination leading to pop-out events is enhanced. This increase in pop-out recombination is independent of an enhancement in intrachromosomal gene conversion events leading to Leu^+Ura^+ recombinants (Tables 2, 3 and 5). Since one of the mechanisms by which a pop-out occurs is reciprocal exchange, this result would be consistent with the suggestion that reciprocal exchange occurs at the G_2 stage of the cell cycle (ROMAN and FABRE 1983). However, the fact that mutations *cdc5* and *cdc14* can also affect intrachromosomal pop-out events (Table 5) makes it difficult to restrict the pop-out events to a specific stage of the cell cycle.

Among the mutations that preferentially enhance gene conversion events, *hpr5* is particularly interesting. The approximately 10-fold increase in recombination events leading to Leu^+ strains in *hpr5* mutants can be attributed to the 10-fold increase in gene conversion events that lead to Leu^+Ura^+ recombinants (Table 3). The gene conversion events in *hpr5* are biased as to which allele is repaired to wild type in the duplication *leu2-112::URA3::leu2-k*. The *leu2-k* allele is converted to wild type much more often in this mutant than in the wild-type strain (Figure 2). The preferential conversion of the *leu2-k* allele over the *leu2-112* could be due to the difference in the nature of both alleles. The *leu2-k* is a deletion of 7 bp (H. KLEIN, unpublished results) while the *leu2-112* is a +1 frameshift mutation (M. CULBERTSON, personal communication). However, sequence specificity for the gene conversion substrate or a different distribution of initiation events as compared to wild type could explain the bias. The lack of post-meiotic segregation in some alleles, such as deletions (FOGEL *et al.* 1978), suggests that DNA heteroduplex mismatches in *S. cerevisiae* may be corrected via more than one mechanism. Studies on meiotic gene conversion at the *b2* locus of *Ascobolus immersus* has led to the suggestion that there are two mechanisms for gene conversion in this organism (HAMZA *et al.* 1986). The *hpr5* mutation may enhance a minor gene conversion pathway in *S. cerevisiae*.

However, the increase in both types of recombination, gene conversion and pop-out, in mutants *hpr2*, *hpr3* and *hpr7*, shows that increased gene conversion and reciprocal exchange can require common functions. This is consistent with the existence of a major pathway for intrachromosomal recombination in which gene conversion and reciprocal exchange are associated.

In spite of the differences in the effect of some of the hyper-recombination mutations on intrachromosomal gene conversion and pop-out events, we have shown for some of the mutants that both types of events require the *RAD52* gene product to express the hyper-recombination phenotype (Table 5). This result confirms that a *RAD52* dependent recombination pathway is being used, whether a hyper-gene conversion or hyper-pop-out phenotype is produced.

There are several ways to achieve a preferential hyper-recombination phenotype for intrachromosomal gene conversion or pop-out recombination. The first is by increased action of a minor pathway for recombination, in which case the requirement for *RAD52* implies an overlap in gene functions. In *A. immersus* two pathways for recombination have already been inferred (HAMZA *et al.* 1986). Second, some step of a major recombination pathway could be affected, changing the outcome of the recombination pathway. The fact that different *hpr* mutations increase different types of recombination events (*hpr1* enhances pop-out recombination, whereas *hpr5* enhances gene conversion) suggests that if there is only one major pathway for intrachromosomal recombination, gene conversion and pop-out recombination can be regulated by different genes that can be defined mutationally.

These mutations should provide useful tools for the further genetic analysis of intrachromosomal recombination. Cloning of the *HPR* genes will lead, we hope, to the identification of gene products involved in the recombination processes.

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