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Increase in incidence of chromosome instability and non-conservative recombination between repeats in *Saccharomyces cerevisiae hpr1Δ* strains

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Abstract Null *hpr1Δ* strains show a large increase (up to 2000-fold) over wild type in the frequency of occurrence of deletions between direct repeats on three different chromosomes. However, we show that *hpr1Δ* mutations have little or no effect on reciprocal exchange, gene conversion or unequal sister chromatid exchange, as determined using intrachromosomal, interchromosomal and plasmid-chromosome assay systems. A novel intrachromosomal recombination system has allowed us to determine that over 95% of deletions in *hpr1Δ* strains do not occur by reciprocal exchange. On the other hand, *hpr1Δ* strains show chromosome loss frequencies of up to 100 times the wild-type level. Our results suggest that yeast cells have a very efficient non-conservative recombination mechanism, dependent on *RAD1* and *RAD52*, that causes deletions between direct DNA repeats, and this mechanism is strongly stimulated in *hpr1Δ* strains. The results indicate that the Hpr1 protein is required for stability of DNA repeats and chromosomes. We propose that in the absence of the Hpr1 protein the cell destabilizes the genome by allowing the initiation of events that lead to deletions of sequences between repeats, and to chromosome instability. We discuss the roles that proteins such as Hpr1 have in maintaining direct repeats and in preventing non-conservative recombination and consider the importance of these functions for chromosome stability.

Key words DNA deletions · Reciprocal exchange Non-conservative recombination · Yeast · *hpr1Δ* mutation

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Introduction

Homologous recombination can occur in mitosis between two homologous DNA sequences independently of their location. Recombination has been reported between homologous DNA sequences located on the same chromosome, on homologous chromosomes, on non-homologous chromosomes, on plasmids, and on a plasmid and a chromosome (for reviews see Petes and Hill 1988; Bollag et al. 1989; Petes et al. 1991).

The outcome of the recombination event varies considerably between different systems. In all cases, crossing-over and gene conversion have been detected. Recombination models propose that there is a common recombination intermediate that gives rise to gene conversion and crossing-over (Holliday 1964; Meselson and Radding 1975; Szostak et al. 1983). This association can be found in almost all yeast recombination systems (for reviews see Fogel et al. 1981; Esposito and Wagstaff 1981; Orr-Weaver and Szostak 1985). Although most gene conversions occurring between intrachromosomal repeats in mitosis (Jackson and Fink 1981) and meiosis (Klein and Petes 1981; Klein 1984; Jackson and Fink 1985) have been reported not to be associated with crossing-over, and reciprocal exchange correlates with long co-conversion events (Ahn and Livingston 1986; Aguilera and Klein 1989b), all results are consistent with the association of both types of recombination.

Nevertheless, recombination between long direct repeats (over 100 bp long) leading to deletions does not completely follow the predictions of homologous recombination models. In yeast, deletions show properties not shared with most reciprocal recombination events detected in intermolecular recombination. They show a low dependency on the *RAD52* gene (Jackson and Fink 1981; Klein 1988; Dornfeld and Livingston 1992) in contrast to the high dependency of intra- or

interchromosomal gene conversion (Malone and Esposito 1980; Jackson and Fink 1981) or reciprocal exchange between inverted repeats (Willis and Klein 1987; Aguilera and Klein 1989; Dornfeld and Livingston 1992). Also, deletions are independent of the length of homology (Yuan and Keil 1990) in contrast to the situation with interchromosomal systems (Strathern et al. 1979). Important alternative mechanisms may lead to deletions between DNA repeats: unequal sister chromatid exchange (SCE), unequal sister chromatid gene conversion (Maloney and Fogel 1987; Rothstein et al. 1987) single strand annealing (SSA) between two ends of the same molecule (Lin et al. 1984; Ozenberger and Roeder 1991; Fishman-Lobell et al. 1992) or half crossing-over (Takahashi et al. 1992).

The use of artificial double strand breaks (DSBs) in the study of recombination is providing insight into the mechanism leading to deletions between direct repeats. Whereas studies of intermolecular recombination induced by a DSB have produced results that can be explained by a DSB repair model (Orr-Weaver et al. 1981, 1988; Ray et al. 1988), studies performed on direct repeat duplications suggest a different explanation. A conventional DSB repair model does not easily explain the following data from yeast: (1) excision recombination is greatly stimulated by a DSB in the nonhomologous intervening sequence (Nickoloff et al. 1989; Rudin and Haber 1988; Rudin et al. 1989); (2) deletions appear earlier than gene conversions in contiguous DNA repeats with an HO cleavage site in between (Fishman-Lobell et al. 1992); and (3) rDNA deletions stimulated by an HO cleavage site are independent of *RAD52* (Ozenberger and Roeder 1991). The repair of DSBs by non-conservative recombination could explain these data, however.

The analysis of mitotic recombination in *hpr1Δ* null strains should help to understand how spontaneous deletions occur between repeats, since the original *hpr1* point mutation shows increased levels of pop-out recombination and almost wild-type levels of gene conversion between repeats (Aguilera and Klein 1988). We show in this study that yeast cells have very active non-conservative mechanisms that cause deletions between direct repeats. These mechanisms are responsible for most of the deletions specifically stimulated in *hpr1Δ* strains, and require the *RAD1* and *RAD52* gene products. Null *hpr1Δ* mutations have very little effect on other types of recombination events (reciprocal exchange, gene conversion, unequal SCE), but there is an increase in the frequency of chromosome loss as compared with the wild-type level. We discuss the importance of proteins such as Hpr1 in the prevention of events that initiate deletions or chromosome loss, as well as the role that direct repeats and non-conservative recombination may play in eukaryotic genome stability.

Materials and methods

Strains

The yeast strains used in this study are listed in Table 1. All strains are genetically related to strains A3Y3A and W303-1A.

Media and growth conditions

Standard media such as YEPD, synthetic complete medium with bases and amino acids omitted as specified, and sporulation medium were prepared according to standard procedures (Sherman et al. 1986). The carbon source used was either 2% glucose or 2% fructose + 0.1% glucose for rich (YEPD or YEPFd, respectively) and synthetic complete media (referred to as SD or SFd). L-Canavanine sulfate and 5-fluoro-orotic acid (5-FOA) were added to synthetic medium at concentrations of 60 and 750 μg/ml, respectively. Occasionally SD + FOA plates were prepared by using 1 g/l proline as nitrogen source and 500 μg/ml FOA. All yeast strains were grown at 30°C with horizontal shaking for liquid cultures. Yeast strains were transformed by the lithium acetate method (Ito et al. 1983).

Determination of recombination rates

Recombination rates were calculated using the median method of Lea and Coulson (1948). Experiments were performed as described previously (Aguilera and Klein 1989a, b). Yeast strains were grown on YEPD, YEPFd or SFd – trp plates as specified, and after 3 days independent colonies were picked, resuspended in water and plated on SD + FOA, SD – trp or YEPD to determine the frequencies of Ura^-, Pgi^+, Trp^- or Ade^- recombinants, respectively. Pgi^+ recombinants were shown to have an unstable Pgi^+ phenotype by virtue of the fact that they carried the gene on an unstable plasmid. Trp^- or Ade^- recombinant colonies were scored after replica-plating from YEPD onto SD – trp or SD – ade medium, respectively.

Determination of chromosome loss frequencies

The frequency of chromosome loss was determined through fluctuation tests performed like those for the recombination rates. The frequency of loss of chromosome V was determined by calculating first the frequency of Can^r segregants. Simultaneously, independent Can^r colonies were isolated to determine the proportion of Hom^- His^- colonies among the Can^r strains. This proportion was multiplied by the frequency of Can^r strains to give the frequency of loss of chromosome V. The frequency of loss of chromosome XV was obtained in a similar way. First the frequency of Ura^- strains was calculated by a fluctuation test performed on SC + FOA plates (the diploids used have a unique *URA3* copy at the *HIS3* locus of chromosome XV). Simultaneously, independent Ura^- colonies were isolated to determine the proportion of Ade^- Leu^- His^- colonies among the Ura^- strains. This proportion was multiplied by the frequency of Ura^- strains to give the frequency of loss of chromosome XV. In the *hpr1Δ/hpr1Δ* mutants, the frequency of loss of chromosome XV was also calculated by a second method. Independent cultures were plated onto YEPD to obtain single colonies. The number of Ade^- Ura^- Leu^- His^- red colonies was then determined and the median frequency value for chromosome loss was obtained directly.

Table 1 Strains

Strain	Genotype	Source
A3Y3A	*MATα ade2-1 ura3-52 his3Δ200 leu2-k::ADE2-URA3::leu2-k*	Aguilera and Klein (1990)
A3Y3T3	*MATα ade2-1 ura3-52 his3Δ200 hpr1Δ3::HIS3 leu2-k::ADE2-URA3::leu2-k*	Aguilera and Klein (1990)
A356-12B	*MATa ura3-52 his3k::LEU2-leu2-r::his3Δ5′-URA3 trp1 leu2Δ68*	Aguilera and Klein (1989)
W303-1A	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	R. Rothstein
AA153-3A	*MATα pgi1-101 ade2 ura3 his3Δ200 trp1 leu2-k*	This study
AA153-3A1	*MATα pgi1-101 ade2 ura3 his3Δ200 trp1 LEU2::pgi1-101-ARS1-TRP1::leu2-k,r*	This study
AA153-7D	*MATa pgi1-101 ade2 ura3 his3Δ200 trp1 hpr1Δ3::HIS3 leu2-k*	This study
AA153-7D3	MAT*a pgi1-101 ade2 ura3 his3Δ200 trp1 hpr1Δ3::HIS3 LEU2::pgi1-101-ARS1-TRP1::leu2-k,r*	This study
AA153-7D4	*MATa pgi1-101 ade2 ura3 his3Δ200 trp1 hpr1Δ3::HIS3 ILEU2::pgi1-101-ARS1-TRP1::leu2-k,r*	This study
AA153-7D6	*MATa pgi1-101 ade2 ura3 his3Δ200 trp1 hpr1Δ3::HIS3 ILEU2::pgi1-101-ARS1-TRP1::leu2-k,r*	This study
AHA-3C	*MATα pgi1-101 ade2 ura3 his3Δ200 hpr1Δ3::HIS3 leu2-k::URA3-ADE2::leu2-k*	This study
XHW-2A	*MATα rad52-1 ade2-1 ura3 his3 trp1 leu2-k::ADE2-URA3::leu2-k can1-100*	This study
XHW-3A	*MATa rad52-1 ade2-1 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-k::ADE2-URA3::leu2-k*	This study
XHW-4C	*MATa rad52-1 ade2-1 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-k:: ADE2-URA3::leu2-k can1-100*	This study
XHY-2C	*MATa rad52-1 ade2 ura3 his3k:: LEU2-leu2-r::his3Δh-URA3 trp1 leu2 can1-100*	This study
XHY-18B	*MATα rad52-1 ade2 ura3 his3k::LEU2-leu2-r::his3Δh-URA3 trp1 hpr1Δ4::TRP1 leu2 can1-100*	This study
XHY-15C	*MATα rad52-1 ade2 ura3 his3k:: LEU2-leu2-r::his3Δh-URA3 trp1 hpr1Δ4::TRP1 leu2 can1-100*	This study
AFA3-6B	*MATα rad1-1 ade2 ura3 his3 trp1 leu2-k::ADE2-URA3::leu2-k*	This study
AFA3-1A	*MATα rad1-1 ade2 ura3 his3 hpr1Δ3::HIS3 leu2-k::ADE2-URA3::leu2-k*	This study
AW115-6C	*MATa ade2 ura3 his3 trp1 leu2-112::URA3::leu2-k*	This study
AW115-14B	*MATa ade2 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-112::URA3::leu2-k*	This study
A647-10B	*MATα ade2 ura3 his3k::LEU2-leu2-r::his3Δ5′-URA3 trp1 hpr1Δ4::TRP1 leu2-k::ADE2::leu2-k*	This study
A647-19D	*Matα ade2 ura3 his3k::LEU2-leu2-r::his3Δ5′-URA3 trp1 hpr1Δ4::TRP1 leu2-k::ADE2::leu2-k*	This study
AAW19-1C	*MATα ade2 ura3 his3k::LEU2-leu2-r::his3Δ5′-URA3 trp1 hpr1Δ4::TRP1 leu2-k::ADE2::leu2-k*	This study
AYW3-4D	*MATa ade2-1 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-3,112 can1 — 100*	This study
AYW3-3A	*MATα ade2 ura3 trp1 leu2-3,112 his3*	This study
AHW-1B	*MATa ade2-1 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-k::ADE2-URA3::leu2-k can1-100*	This study
AHW-6B	*MATα ade2-1 ura3 his3 trp1 leu2-k::ADE2-URA3::leu2-k can1-100*	This study
AHW-2A	*MATa ade2-1 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-k::ADE2-URA3::leu2-k can1-100*	This study
H52-1A	*MATα ade2 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-k::ADE2-URA3::leu2-k rad1-1 rad52-1*	This study
H52-1B	*MAT a ade2 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-k::ADE2-URA3::leu2-k*	This study
H52-1D	*MATα ade2 ura3 his3 trp1 hpr1Δ3::HIS3 leu2-k::ADE2-URA3::leu2-k rad1-1 rad52-1*	This study
ACYW19-6B	*MAT a leu2-3,112 ura3 his3 trp1 ChrIV::his3Δ3′::his3Δ5′-URA3 hpr1Δ4::TRP1*	This study
ACYW19-6C	*MATα ura3 his3 trp1 ChrIV::his3Δ3′::his3Δ5′-URA3*	This study
ACYW19-2C	*MATα leu2-3,112 ura3 his3 trp1 ChrIV::his3Δ3′::his3Δ5′-URA3*	This study
ACYW19-7A	*MAT a ura3 his3 trp1 ade2 ChrIV::his3Δ3′::his3Δ5′-URA3 hpr1Δ4::TRP1*	This study
HAFG-1B	*MAT a ura3 trp1 leu2 HIS3::leu2-r-LEU2::his3Δh-URA3 hpr1Δ4::TRP1*	This study
HAFG-7B	*MATα ura3 trp1 leu2 HIS3::leu2-r-LEU2::his3Δh-URA3 hpr1Δ4::TRP1*	This study
HAFG-9A	*MATα ura3 trp1 leu2 HIS3::leu2-r-LEU2::his3Δh-URA3*	This study
HAFW-1C	*MATα leu2 ade2 his3 ura3 can1-100 hom3 his1 trp1 hpr1Δ4::TRP1*	This study
HAFW-10C	*MATα leu2 ade2 his3 ura3 can1-100 hom3 his1 trp1 hpr1Δ4::TRP1*	This study
HAFW-13A	*MAT a leu2 ade2 his3 ura3 can1-100 hom3 his1 trp1*	This study
HAFW-5C	*MAT a leu2 ade2 his3 ura3 can1-100 hom3 his1 trp1*	This study

Plasmid constructions

Plasmid pAA403 was made by inserting a 6 kb *Bam*HI-*Hin*dIII fragment containing the *HPR1* gene from plasmid YCpA13 (Aguilera and Klein 1990) into pBR322 to yield plasmid pBR-BH3. From this plasmid we isolated the 4.2 kb *Bgl*II-*Hin*dIII and the 3.3 kb *Eco*RI-*Hin*dIII fragments. Both fragments were ligated with a 0.8 kb *Bgl*II-*Eco*RI YRp7 fragment containing the *TRP1* gene to yield plasmid pAA403. Therefore, in the pAA403 plasmid, the internal 1.2 kb *Bgl*II-*Eco*RI *HPR*1 sequence is replaced by a 0.8 kb *Bgl*II-*Eco*RI YRp7 fragment containing the *TRP*1 gene.

Plasmid pHSR4 was made by subcloning the 6.1 kb *Bam*HI-*Hin*dIII *HPR1*-containing fragment from plasmid YCpA13 into plasmid pRS316 (Sikorski and Hieter 1989).

Plasmid pAA547 was constructed by inserting the 1.45 kb *Eco*RI fragment containing the *TRP1-ARS1* sequences into the *Eco*RI site of plasmid pAA2-7, which consists of the 2.16 kb *Sal*I-*Xho*I *LEU2* fragment containing the *leu2-r* mutation and pBR322.

Plasmid pAA63-11 was constructed by inserting the 6.3 kb *Bgl*II-*Hin*dIII *PGI1* region containing the *pgi1-101* allele into plasmid pAA547 opened at the *Bam*HI and *Hin*dIII sites by partial digestion. The 6.3 kb fragment was obtained from plasmid p25PC, which corresponds to plasmid p25 with the *LEU2* gene replaced by the *pgi1-101* mutation by *in vivo* gene conversion in yeast (Aguilera 1988).

Construction of an *hpr1Δ4::TRP1* deletion strain

Plasmid pAA403 was used to isolate the 3.9 kb *Bam*HI-*Bgl*II fragment containing the *hpr1Δ4::TRP1* construct, by performing

partial digestion with *Bgl*II. This linearized fragment was used to transform strain AA064-8A. Twelve stable Trp^+ transformants were selected and subjected to Southern analysis. By using different probes based on the *HPR1* and *TRP1* genes, we demonstrated that all of them carried the chromosomal *HPR1* gene correctly replaced by the *TRP1* gene (data not shown). This new deletion removes 55% of the *HPR1* coding region and shows an identical recombination phenotype to the previously reported *hpr1Δ3::HIS3* deletion (see below).

Construction of a direct repeat recombination system to detect deletions and reciprocal exchange

To construct this system in the chromosome, strains AA153-3A (*HPR1*) and AA153-7D (*hpr1Δ*) were transformed with plasmid pAA63-11. Unstable Trp^+ transformants carrying a non-integrated replicative pAA63-11 plasmid were selected. From these, stable Leu^+ Trp^+ recombinants were obtained by selection on SD-leu-trp medium. One wild-type (AA153-3A1) and three *hpr1Δ* recombinants (AA153-7D3, AA153-7D4 and AA153-7D6) containing the plasmid pAA63-11 integrated into the *leu2-k* locus were selected for further studies. All of them were shown by Southern analysis to contain a 2.16 kb direct duplication with an 11.9 kb intervening sequence containing the *ARS1*, *TRP1* and *pgi1-101* allele sequences, as shown in Fig. 2.

In these new strains a reciprocal exchange leading to a chromosomal deletion of the duplication system should generate a circular plasmid similar to pAA63-11, the only difference being that it does not necessarily need to carry the *leu2-k* allele of the *LEU2* gene. Since this plasmid contains an *ARS1* sequence and the leaky *pgi1-101* allele, we expected that after several mitotic divisions some cells would have accumulated an increasing number of plasmids owing to missegregation of YRp plasmids in mitosis (Murray and Szostak 1983). In contrast to cells carrying one copy of the *pgi1-101* allele, cells containing numerous copies of the *pgi1-101* allele ferment glucose and are able to grow on synthetic medium containing glucose as the sole carbon source (Aguilera 1988).

To confirm that after one excision event plasmids can accumulate at a concentration high enough to allow detection of a significant proportion of excision events, we performed a reconstruction experiment. Four different yeast transformants carrying plasmid pAA63-11 were plated on selective medium SF-trp, on which cells with one copy of the plasmid can grow. Six colonies of each transformant were obtained from these plates, resuspended in water and plated onto YEPD, where only cells carrying several copies of plasmid pAA63-11 can grow. We found that an average of 9.5% of the total cells could grow on glucose. We also determined that the stability of *ARS1*-based plasmids is the same in *hpr1Δ* and *HPR1* strains (data not shown). Therefore, the system is sensitive enough to detect a significant proportion of reciprocal excision events.

DNA manipulation

Plasmid DNA was isolated from *Escherichia coli* by CsCl gradient centrifugation as described (Clewell and Helinski 1970). Small-scale plasmid DNA preparations were made according to Bolivar and Backman (1979). Yeast genomic DNA was prepared from 5 ml YEPD cultures according to Sherman et al. (1986). Plasmid yeast DNA was prepared according to Hoffman and Winston (1987) and used directly to transform *E. coli*. Yeast chromosomes were isolated and run in contour clamped homogeneous electric field (CHEF) gels as described (Gerring et al. 1991)

[^{32}P]dCTP and digoxigenine-dUTP (Boehringer)-labeled DNA probes 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 6.0 and 30 mM $Na_2P_2O_7$ at 65°C for 18 h when using [^{32}P]dCTP, or in 50% formamide 5 × SSC, 0.01% N-laurosylsarcosine, 0.02% SDS and 2% Boehringer Mannheim blocking reagent at 42°C for 18 h when using digoxigenin-dUTP.

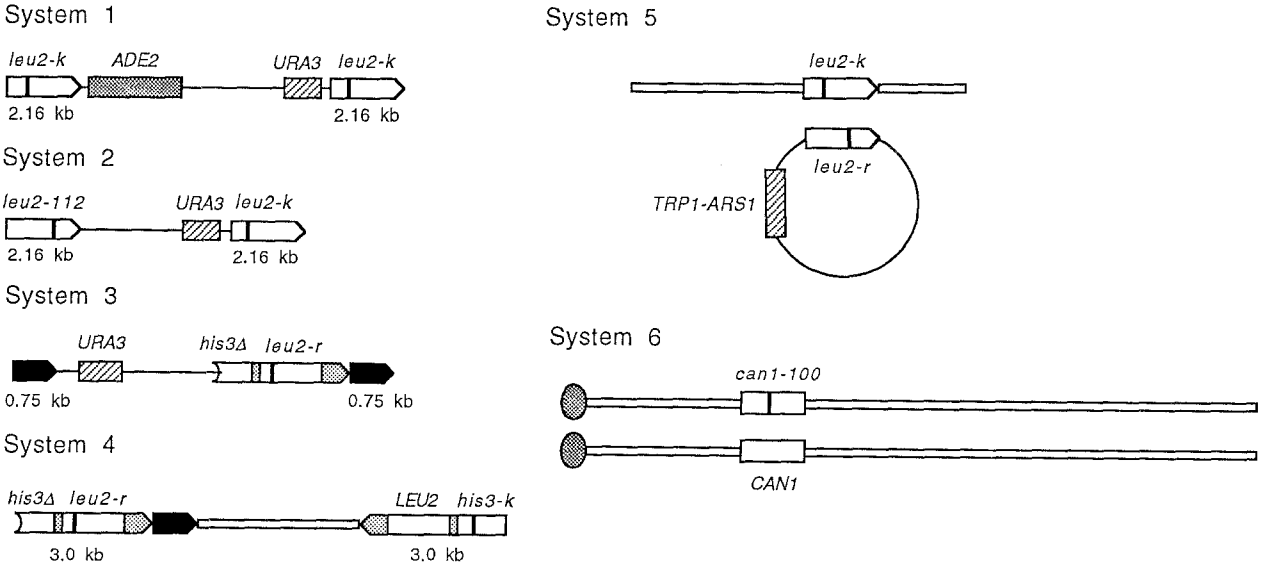


Fig. 1 DNA repeat duplication systems used to characterize the *hpr1Δ* mutation. Systems 1 and 2 carry the 2.16 kb *Sal*I-*Xho*I *LEU2* fragment as the direct repeat (Aguilera and Klein 1989a). Systems 3 and 4 are overlapping and are contained in the *his3-k-LEU2::leu2-r-his3Δ5'::URA3* construction. System 3 is based on a 0.75 kb *Bam*HI-*Bam*HI fragment (*filled block*) from the *HIS3* region and System 4 is based on a 3.0 kb inverted repeat made from the *LEU2* and *HIS3* alleles. System 4 is based on the 2.16 kb *Sal*I-*Xho*I *LEU2* fragment, with the *leu2-r* allele in plasmid pAA547, and the *leu2-k* allele in the natural chromosomal locus. The orientation is indicated only for the repeats. The size of each repeat is given, except for System 6, which represents the same arm of two chromosomes V of a diploid strain. A *vertical line* inside mutant alleles indicates the approximate site of the mutation. *Thin blocks* indicate yeast DNA sequences. *Lines* indicate pBR322 sequences. *Dotted regions* in Systems 4 and 5 indicate *HIS3* regions other than the *HIS3* open reading frame

Detection of digoxigenin-labeled DNA was performed following Boehringer-Mannheim recommendations.

Linear DNA fragments were recovered directly from agarose gels and used in DNA labeling experiments or in ligation reactions with T4 DNA ligase overnight at 14°C.

Results

Null *hpr1* mutants show a very strong increase in the incidence of deletions between DNA repeats and little or no effect on other recombination events

We used two different *hpr1Δ* null mutations in this work: the previously described *hpr1Δ3::HIS3* mutation (see Aguilera and Klein 1990) and the *hpr1Δ4::TRP1* mutation, constructed for this study (see Materials and methods). We determined the effects of these null mutations on the incidence of (1) deletions between direct repeats located in three different chromosomes, (2) intrachromosomal gene conversion, (3) reciprocal exchange and gene conversion on inverted repeats, (4) recombination between a plasmid and a chromosome, and (5) recombination between homologous chromosomes.

Deletion events between direct repeats were detected with three different systems (Fig. 1). System 1 (*leu2-k::ADE2-URA3::leu2-k*) and System 2 (*leu2-112::URA3::leu2-k*) are based on a 2.16 kb direct repeat located at the *LEU2* locus on chromosome III (Aguilera and Klein 1989a). System 3 (carried by the *his3-k-LEU2::leu2-r-his3Δ5′::URA3* construct) is based on a 0.75 kb direct repeat located in the *HIS3* region from chromosome XV (Aguilera and Klein 1989b). The three systems contain the *URA3* gene in the sequence located between the direct repeats. Deletion events were scored as Ura^- colonies on SD + FOA plates.

Intrachromosomal gene conversion was determined with System 2 by the identification of Leu^+ Ura^+ recombinants on SD-leu-ura plates.

Intrachromosomal reciprocal exchange and gene conversion was determined with System 4 (carried by the *his3-k-LEU2::leu2-r-his3Δ5′::URA3* construct), which is based on a 3.0 kb inverted repeat located in the *HIS3* region on chromosome XV (see Aguilera and Klein 1989b). Recombinants were scored as His^+ colonies on SD-his. With this system we could determine the proportion of reciprocal exchanges among all recombination events, because about 40% of the His^+ recombinants carried an inversion of the system, as expected for a reciprocal exchange event (Aguilera and Klein 1990).

Recombination between a plasmid and a chromosome was determined between a 2.16 kb *LEU2* fragment (*leu2-r*), located on a replicative plasmid (pAA547), and the natural *LEU2* locus (*leu2-k*) on chromosome III (System 5, Fig. 1). Recombinants were scored as Leu^+ colonies on SD-leu. Reciprocal exchange and gene conversion could be distinguished because a reciprocal exchange integrates the plasmid

Table 2 Frequency of recombination ($\times 10^6$) in *HPR1* and *hpr1Δ* strains assayed using the six different recombination systems shown in Fig. 1

Type of event[a]	System[b]	Recombinant phenotype	Strain[c] *HPR1*	Strain[c] *hpr1Δ*	
Intrachromosomal					
Deletions	1	Ura^-	20	19 000	(×950)[d]
	2	Ura^-	360	97 000	(×270)
	3	Ura^-	250	109 000	(×436)
G.C.	2	Leu^+ Ura^+	40	380	(×9.5)
	5	His^+	72	96	(×1.3)
R.E.	4	His^+	48	64	(×1.3)
Plasmid-chromosome					
G.C.	5	Leu^+ Trp^+(i)	12	16	(×1.3)
R.E.	5	Leu^+ Trp^+(e)	18	24	(×1.3)
Interchromosomal					
R.E. + G.C.	6	Can^r	70	140	(×2)

[a] G.C. gene conversion; R.E. reciprocal exchange

[b] Systems are those shown in Fig. 1. For System 4 the distinction between R.E and G.C was made by Southern analysis. For system 5, Trp^+(e) and Trp^+(i) indicate stable and unstable Trp^+ phenotypes, respectively

[c] Strains used for each system were: System 1, A3Y3A (*HPR1*) and A3Y3T3 (*hpr1Δ*); System 2, AW115-6C (*HPR1*) and AW115-14B (*hpr1Δ*); Systems 3 and 4, A356-12B (*HPR1*), AA647-19D (*hpr1Δ*) and AA647-10B (*hpr1Δ*); System 5, pA547 Trp^+ transformants of strains AHW-6B (*HPR1*), AHW-1B (*hpr1Δ*) and AHW-2A (*hpr1Δ*); System 6, diploids AYW3-3A/W303-1A (*HPR1/HPR1*) and AYW3-4D/AAW19-4D (*hpr1Δ/hpr1Δ*). For each strain, six different and independent colonies were used for a fluctuation test. A median frequency was obtained for each strain, and in cases where more than one strain was used, only the value of one is given. No significant variation (<15%) was observed with the rest

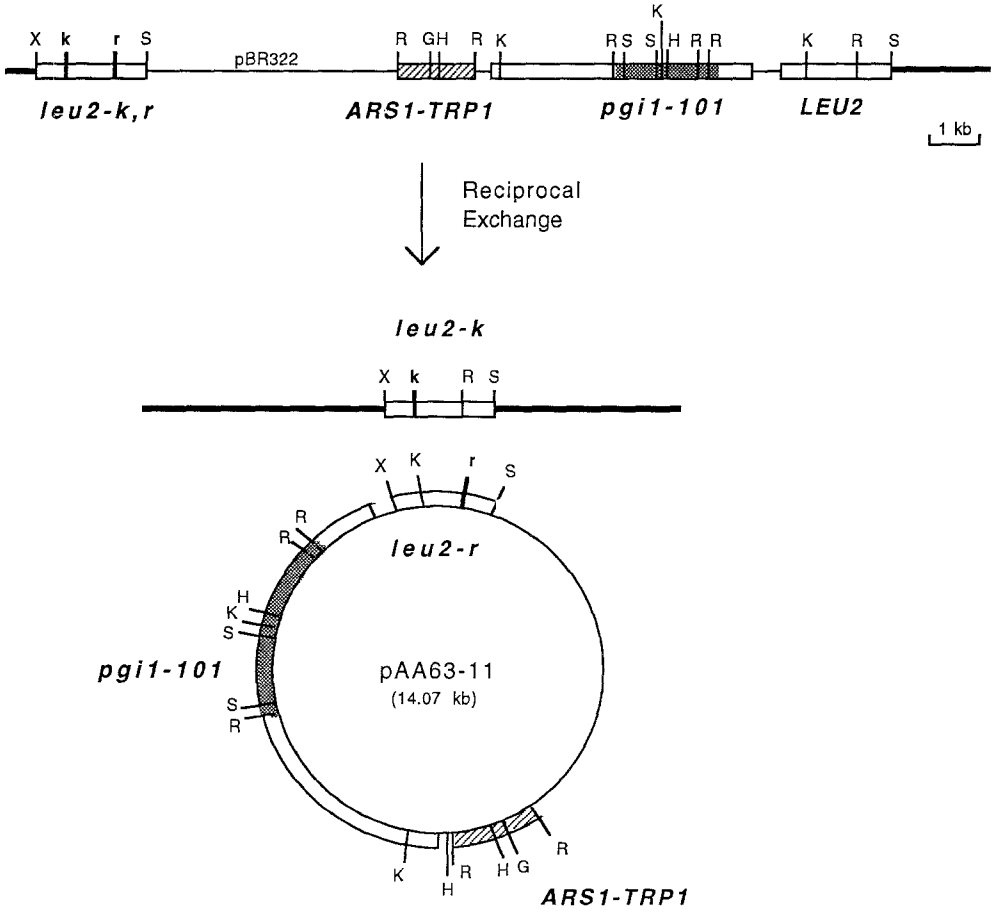
[d] Numbers in parentheses indicate the relative increase over the wild-type value

into the chromosome. Those Leu$^+$ recombinants stable for the phenotype provided by the plasmid pAA547 (Trp$^+$) were scored as reciprocal exchange and those that were unstable as gene conversion events.

Recombination between homologous chromosomes (reciprocal exchange and gene conversion) was determined in *can1-100/CAN1* diploid strains (System 6). Recombinants were scored as Can^r colonies on SD + can.

Table 2 shows that *hpr1Δ* null mutants have a very high frequency of deletions between direct repeats (270–950 times the wild-type levels) in the three systems tested (1, 2 and 3). However, intrachromosomal gene conversion, determined with system 2, was much less affected (9.5 times the wild-type levels). These results might suggest that *HPR1* specifically controls reciprocal exchange (as the mechanism responsible for deletions between direct repeats) and has a relatively small effect on gene conversion. This does not seem to be the case, because we find no difference between *HPR1* and *hpr1Δ* strains (1.3-fold difference) with systems 4 and 5, which measure both gene conversion and reciprocal exchange. The lack of a strong effect on gene conversion and reciprocal exchange was further corroborated by measurements of recombination between homologous chromosomes (System 6). The *hpr1* null mutants and the wild-type strains have similar levels of mitotic recombination between homologous chromosomes (2-fold difference). Therefore, the *HPR1* gene is essentially involved in ensuring low levels of deletions between repeats and has little effect on reciprocal exchange.

Fig. 2 Direct repeat recombination system constructed with plasmid pAA63-11 to detect reciprocal exchange between repeats (see Materials and methods). Cells carrying this system and the *pgi1-101* allele (which provides 2% of wild-type PGI levels) at the original chromosome II *PGI* locus are unable to grow on 2% glucose media (Pgi$^-$ phenotype). Reciprocal exchange between the *leu2* repeats leads to the deletion of the whole system from the chromosome and to the generation of a replicative plasmid containing the *pgi1-101* allele. This plasmid can accumulate in the cell to a concentration sufficient to increase the cellular PGI activity to levels (20%–30% wild-type value) that permit cell growth on 2% glucose medium (Pgi$^+$ phenotype; see text). Only one case of reciprocal exchange is shown—that leading to the *leu2-r* allele on the plasmid and the *leu2-k* allele on the chromosome. However, four types of alleles can be found on each carrier (*leu2-k*, *leu2-r*, *leu2-k,r* or *LEU2* alleles). Also, the chromosomal system shown is that of strains AA153-7D4 and AA153-7D6. Strains AA153-3A and AA153-7D3 carry a *leu2-r* allele on the left side. (Restriction sites shown are: *B* *Bam*HI, *G* *Bgl*II, *H* *Hin*dIII, *K* *Kpn*I, *R* *Eco*RI, *X* *Xho*I, *Xb* *Xba*I) A *small letter* indicates that the restriction site was mutated by Klenow reaction



Deletions in *hpr1Δ* strains do not occur through a reciprocal recombination mechanism

The results show that the *HPR1* gene has no effect on either intra- or inter-chromosomal homologous recombination events that occur through a mechanism associated with gene conversion and reciprocal exchange. The deletion events observed in *hpr1Δ* strains occur either through a different type of reciprocal exchange mechanism, or through non-conservative recombination. In order to test these hypotheses we constructed a novel chromosomal recombination system that allows simultaneous detection of both products of the reciprocal exchange event: the chromosomal deletion and the formation of a stable circular plasmid (Fig. 2).

In this direct repeat system, *LEU2::pgi1-101-ARS1-TRP1::leu2-k*, a reciprocal exchange leading to a deletion should generate a circular plasmid similar to pAA63-11, with the only difference being that it would not necessarily contain the *leu2-k* allele. This plasmid contains an *ARS1* sequence and the leaky allele *pgi1-101*. We expected that after several mitotic divisions some cells would have accumulated an increased number of plasmids owing to missegregation of YRp plasmids in mitosis (Murray and Szostak 1983). In contrast to cells carrying one copy of the *pgi1-101* allele, which are phenotypically Pgi^-, cells containing numerous copies of the *pgi1-101* allele, are Pgi^+: they ferment glucose and are able to grow on synthetic medium containing glucose as the sole carbon source (Aguilera 1988). In reconstruction experiments (see Materials and methods) we have determined that in 9.5% of cells transformed with replicative plasmid pAA63-11, the plasmid accumulates to levels high enough to produce a Pgi^+ phenotype.

Before determination of the frequency of reciprocal exchange, we confirmed that Pgi^+ recombinants contained the expected circular plasmid. First, we showed that all putative recombinants were unstable for the Pgi^+ phenotype: after a round of growth on permissive YEP-2% fructose medium, > 80% of colonies lost the ability to grow on YEP-2% glucose. And finally, plasmids were isolated from 29 yeast recombinants, propagated in *E. coli* and characterized by restriction analysis (Fig. 3).

The results for the frequency of Pgi^+ recombinants in Hpr^+ and *hpr1Δ* strains are shown in Table 3. Pgi^+ reciprocal exchange events occur in *hpr1Δ* strains at a frequency 5-23 times higher than in $Hpr1^+$ strains (either *HPR1* strains or *hpr1Δ* strains containing the centromeric plasmid pHSR4 carrying the functional *HPR1* gene). The frequency of deletion of the DNA repeats in the chromosome was three orders of magnitude higher in *hpr1Δ* than in $Hpr1^+$ strains. These results clearly show that the effect of the *hpr1Δ* mutation on reciprocal exchange between repeats is much less significant than its effect on deletion formation. This suggests that the majority of deletion events

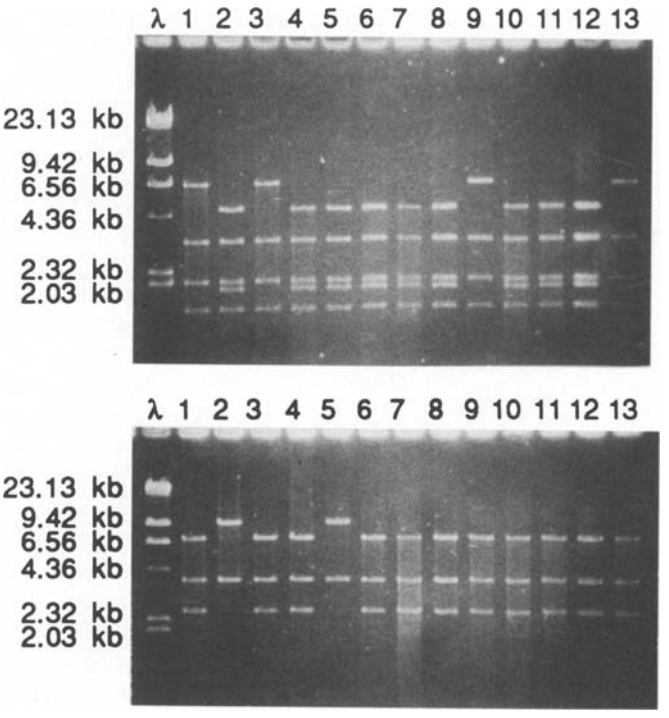


Fig. 3 Ethidium bromide-stained agarose gel of plasmid DNA isolated from Pgi^+ recombinants and digested with *Eco*RI (top) or *Kpn*I (bottom). Lanes 1 and 13, pAA63-11 (carrying the *leu2-r* allele); lane 2, pAA62-12 (as pAA63-11 but with the *leu2-k* allele); lane 3, plasmid isolated from a AA153-7D6 recombinant; lanes 4-6, plasmids from AA153-7D3 recombinants; lanes 7-9, from AA153-7D4; lanes 10-12, from AA153-3A1

(> 99%) in *hpr1Δ* strains do not involve reciprocal exchange between the repeats. The results suggest that a recombination mechanism different from that which gives rise to reciprocal exchange leads to deletions in *hpr1Δ* strains.

The same conclusion can be reached by noting the percentage of deletions due to reciprocal exchange (Table 3). This percentage can be calculated by dividing the frequency of reciprocal exchange by the frequency of deletions and multiplying this value by 9.5% (our system detects at most 9.5% of total reciprocal exchanges between repeats; see Materials and methods). We find that, in $Hpr1^+$ strains (either *HPR1* or *hpr1Δ* transformed with pHSR4), at least 6.6% of the deletions occur through reciprocal exchange, but in *hpr1Δ* strains this value goes down to 0.07%. The result clearly suggests that reciprocal exchange is not the predominant mechanism leading to deletions in *hpr1Δ* strains.

Unequal SCE is not affected in *hpr1Δ* strains

A mechanism leading to deletions between DNA repeats that could explain the lack of effect of the *hpr1Δ* mutation on reciprocal exchange and gene conversion

Table 3 Frequency of deletions and reciprocal exchanges associated with deletions in *HPR1* and *hpr1Δ* strains in the *leu2-k::pgi1-101-ARS1-TRP1::leu2-k* system

Strain	Reciprocal exchange[a] ($\times 10^6$)	Deletions[b] ($\times 10^6$)
HPR1		
A3Y3A	–	30 (×1)[c]
AA153-3A1	0.2 (×1)[c]	–
AA153-7D3 [*HPR1*]	0.2 (×1)	–
AA153-7D4 [*HPR1*]	0.5 (×2.5)	–
hpr1Δ		
AA153-7D3	2.3 (×11.5)	43000 (×1433)
AA153-7D4	1.0 (×5)	40000 (×1333)
AA153-7D6	4.6 (×23)	63000 (×2100)

[a] Reciprocal exchange frequencies were determined by selecting Pgi^+ recombinants on synthetic-2% glucose medium. For each strain six different, independent colonies were used for a fluctuation test. The median frequency is given as calculated by Lea and Coulson (1948)

[b] Deletion frequencies were obtained by determining the number of Trp^- colonies among total Colonies, except for the wild-type strain A3Y3A, whose value corresponds to the frequency of Ura^- deletions obtained with the *leu2-k::URA3-ADE2::leu2-k* system. This exception was necessary because the low frequency of deletions in the wild-type strains makes it necessary to isolate more than 10^7 cells to obtain a reliable result based on the Trp^- phenotype. In order to confirm that the deletion frequency calculated from the Trp^- phenotype was indistinguishable from that calculated from the Ura^- phenotype, we constructed diploids with the listed strains and AHA-3C (*hpr1Δ*) carrying the *leu2-k::URA3-ADE2::leu2-k* system. In the three diploids tested, similar increases in deletion formation over the wild-type value were observed: ×2222 (AA153-7D3), ×2777 (AA153-7D4) and ×1916 (AA153-7D6)

[c] Numbers in parentheses indicate the relative increase over the wild-type value

between repeats is unequal SCE. To test whether deletions in *hpr1Δ* strains occurred through unequal SCE, the A3Y3T3 *hpr1Δ* strain carrying the *leu2-k::URA3-ADE2::leu2-k* system was used to obtain half sectored colonies (red: white) with which we could determine whether the deletion carried by the red half (loss of *ADE2*) of the colony was accompanied by a triplication in the white part of the colony as expected for an unequal SCE event. We found 31 half sectored colonies among a total of 7650 colonies isolated on YEPD plates. Southern blot analysis of the white sector of 29 colonies showed that 27 contained the same original duplication system, and only 2 contained a triplication. These results suggests that unequal SCE does not account for the vast majority of the deletion events in *hpr1Δ* strains.

However, although most of the deletion events observed in *hpr1Δ* strains do not occur through an unequal SCE mechanism, this result does not imply that the *hpr1Δ* mutation has no effect on unequal SCE. To determine this, we used the direct repeat system described by Fasullo and Davis (1987) to score unequal SCE. This sytem (denoted in this work as ChrIV: *his3Δ3'::his3Δ5'-URA3*) is based on a direct repeat of a 300-bp *HIS3* fragment on chromosome IV (Fig. 4). Unequal SCE can be scored as His^+ recombinants on SD-his. As an internal control for deletions, we scored Ura^- events on SD + FOA. These events occur owing to a deletion between the two direct repeats created by the chromosome IV DNA fragment used to integrate the system on the chromosome. Table 4 shows that in *hpr1Δ* strains unequal SCE was little affected (2.1 to 2.9-fold) whereas the incidence of deletions was greatly increased (1560 to 3960-fold) as compared with the wild-type values. Therefore unequal SCE is not affected by the *hpr1Δ* mutation.

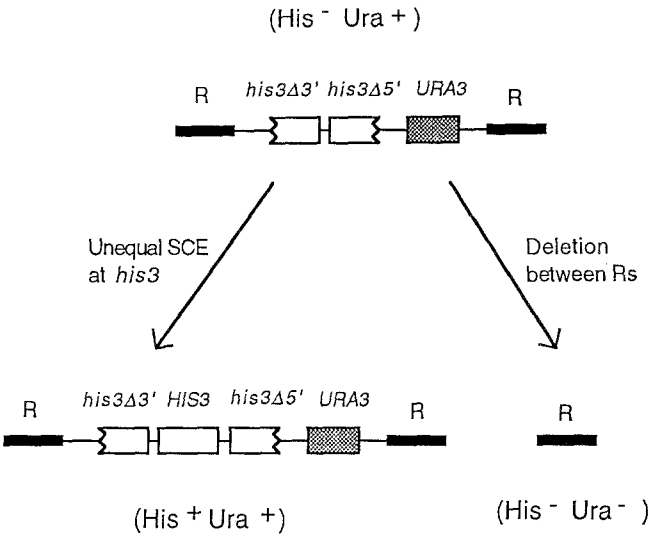


Fig. 4 Diagram of the system used to detect unequal sister chromatid exchange (Fasullo and Davis 1987) showing the genetic structure and the corresponding phenotype (*in parentheses*) of unequal SCE and deletion events determined in our experiments (see Table 5). *R* indicates the DNA region from chromosome IV that was used to integrate the system into the chromosome

Table 4 Frequency of unequal SCE in *HPR1* and *hpr1Δ* strains

Strain	Frequency of unequal SCE-($\times 10^6$)[a]	Frequency of deletions ($\times 10^6$)[a]
HPR1		
ACYW19-6C	9.0	40
ACYW19-2C	5.4	10
hpr1Δ		
ACYW19-6B	20.0 (×2.8)[b]	99000 (×3960)[b]
ACYW19-7A	15.0 (×2.1)[b]	39000 (×1560)[b]

[a] Unequal SCE was scored as the His^+ phenotype and deletions as the Ura^- phenotype. For each strain, six different and independent colonies were used for a fluctuation test. The median frequency is given as calculated by Lea and Coulson (1948)

[b] Numbers in parentheses indicate the relative increase over the wild-type value. The reference wild-type value used was the average of the two median frequencies for each type of event

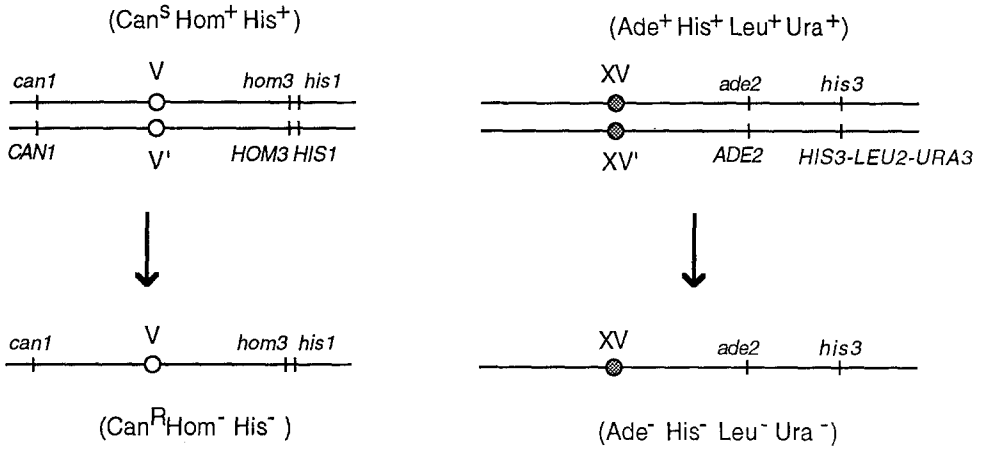


Fig. 5 Diagram of the original set of chromosomes V and XV used to score chromosome loss. The loss of the chromosome marked as V' or XV' would lead to cells carrying only the chromosome marked as V or XV. The phenotypes determined by each set of chromosomes are shown in *parentheses*. Two versions of chromosome XV' were used: one carrying the inverted repeat construct *his3-k::LEU2-leu2-r::his3Δ5'-URA3* at the *HIS3* locus (this is the case diagrammed in this Figure), and a second containing the *LEU2* gene inserted next to a *his3-k* allele at the *HIS3* locus. The results were the same in both cases (see Table 5)

Chromosome loss in *hpr1Δ* strains

The specific effect of *hpr1Δ* on deletions between DNA repeats could be a consequence of a high incidence of DNA lesions or breaks. This would imply that breaks occurring between direct DNA repeats can be efficiently repaired by an intrachromatid, non-conservative mechanism. Otherwise, either the DNA break is repaired by interchromatid or interchromosomal recombination or it leads to chromosome instability. For this reason, we determined the frequency of loss for chromosomes V and XV (Fig. 5) in *hpr1Δ/hpr1Δ*, *HPR1/hpr1Δ* and *HPR1/HPR1* diploid strains as described in Materials and methods. Table 5 shows that mutant *hpr1Δ/hpr1Δ* diploid strains have a higher frequency of chromosome loss than wild-type strains (over 100-fold for chromosomes XV and V). A number of diploid segregants genetically identified as monosomic for chromosome XV were analyzed by CHEF electrophoresis and tetrad analysis to confirm that they were monosomic. The CHEF electrophoresis pattern of the chromosomes of eight tested strains showed that they did not arise by chromosome translocation or long DNA deletions (data not shown). The tetrad analysis of 11 putative monosomic strains suggested that most of the *hpr1Δ* events involved chromosome loss: of the 11 putative monosomic strains studied, 6 gave tetrads with the expected ≤ 2 viable: ≥ 2 non-viable segregation, and 5 gave 2 tetrads each with 4 viable spores, from a total of 5 tetrads analyzed for each putative monosomic strain. As suggested by Hartwell and Smith (1985) these latter cases could have occurred by mitotic nondisjunction that restored disomy. Euploids may have a considerable growth advantage over monosomes and would be strongly selected.

The frequency of loss of chromosome XV was the same regardless of whether two inverted repeats were inserted at the *HIS3* locus or not. This suggests that the Hpr1 protein does not function specifically on DNA repeats (Table 5).

Table 5 Frequency of chromosome loss ($\times 10^6$) in *HPR1* and *hpr1Δ* strains

Diploid[a]	Chromosome V		Chromosome XV[b]	
	Frequency	Loss/Can^r	Frequency	Loss/Ura$^-$
HPR1/HPR1	0.08	1/473	8.0	16/102
HPR1/hpr1Δ	0.26 (×3)	3/509	16.0 (×2)	19/118
hpr1Δ/hpr1Δ	14.3 (×179)	31/498	682.0 (×85)	13/40
hpr1Δ/hpr1Δ	26.1 (×326)	59/532	2378.3 (×297)	16/37

[a] Diploid strains used were HAFG-5A × HAFG-9A (*HPR1/HPR1*), HAFG-7B × HAFW-13A (*HPR1/hpr1Δ*), HAFW-1C × HAFG-1B (*hpr1Δ/hpr1Δ*) and HAFW-10C × HAFG-1B (*hpr1Δ/hpr1Δ*)

[b] For each diploid strain, six independent colonies were used for a fluctuation test. From each strain a median frequency value was obtained. Chromosome loss events were scored as the Can^r Hom$^-$ His$^-$ phenotype for chromosome V and as the Ura$^-$ Leu$^-$ His$^-$ Ade$^-$ phenotype for chromosome XV (see Fig. 5), following primary selection of colonies on SD + can and SD + FOA plates, respectively. The frequency of loss for chromosome XV as determined by the frequency of red colonies formed on YEPD plates was the same (data not shown). The complete analysis was repeated in the same strains without the inverted repeat system at the *HIS3* locus, and the results were the same (data not shown)

Dependence of the hyper-deletion phenotype on *RAD1* and *RAD52* genes

We have determined the dependence of the mechanism that produces deletions of DNA repeats in *hpr1Δ* strains on *RAD1* and *RAD52*. The frequency of Ura$^-$ events (deletions) was determined for System 1 in double mutants carrying one of the *rad* mutations mentioned and the *hpr1Δ* mutation. Table 6 shows that

Table 6 Frequency of recombination of *hpr1Δ* strains on different *rad* mutant backgrounds

Genotype[a]	Frequency of deletions ($\times 10^6$)[b]
System 1	
HPR1	50 (×1)[c]
HPR1 rad52-1	25 (×0.5)
HPR1 rad1-1	100 (×2)
HPR1 rad1-1 rad52-1	6 (×0.1)
hpr1Δ	20000 (×400)
hpr1Δ rad52-1	700 (×14)
hpr1Δ rad1-1	1340 (×27)
hpr1Δ rad1-1 rad52-1	7 (×0.1)
System 3	
HPR1	160 (×1)[c]
HPR1 rad52-1	13 (×0.1)
hpr1Δ	81000 (×506)
hpr1Δ rad52-1	170 (×1)

[a] Systems are those shown in Fig. 1 and recombinants were scored based on the Ura$^-$ phenotype. Strains used for System 1 were A3Y3A (*HPR1*), XHW2A (*HPR1 rad52-1*), AFA3-6B (*HPR1 rad1-1*), H52-1A transformed with centromeric plasmid YCpA13 containing the *HPR1* gene (*HPR1 rad1-1 rad52-1*), H52-1B (*hpr1Δ*), XHW-3A and XHW-4C (*hpr1Δ rad52-1*), AFA3-1A (*hpr1Δ rad1-1*), and H52-1A and H52-1D (*hpr1Δ rad1-1 rad52-1*). Strains used for System 3 were A356-12B (*HPR1*), XHY-2C (*HPR1 rad52-1*) AA647-19D (*hpr1Δ*), and XHY-18B and XHY-15C (*hpr1Δ rad52-1*)

[b] For each strain six different and independent colonies were used for a fluctuation test. A median frequency was obtained for each strain. In cases where two strains were used for a particular genotype, only the value of one is given. An insignificant variation was obtained for the value of the other strain. Deletion recombinants were scored on SD + FOA plates

[c] Numbers in parentheses indicate the relative increase over the wild-type value

the deletions formed in *hpr1Δ* strains depend on the *RAD1* and *RAD52* genes. This dependence, however, is only partial. The double mutant strains *hpr1Δ rad1-1* and *hpr1Δ rad52-1* both show a hyper-deletion phenotype (Table 6). They have higher frequencies of deletions than either *rad1-1* (14-fold) or *rad52-1* (28-fold) single mutants. It is interesting that in *hpr1Δ* strains there is a significant increase over wild-type levels of deletion events that are *RAD52* independent. We confirmed this result with System 3: the double mutant strain *hpr1Δ rad52-1* shows an elevated frequency of deletions (13-fold) when compared with the *rad52-1* single mutant (Table 6). This suggests that a number of the deletions that occur between DNA repeats do not arise by means of the *RAD52* recombinational repair pathway. However, the mechanism is recombinational, because the hyperdeletion phenotype is completely abolished in *hpr1Δ rad1-1 rad52-1* strains.

Discussion

In this paper we show that: (1) deletions between direct repeats are specifically and strongly stimulated in *hpr1Δ* strains at various chromosomal locations; (2) these deletions occur mainly through a non-conservative recombination mechanism; (3) no other type of homologous recombination—intrachromosomal, inter-chromosomal or plasmid-chromosome reciprocal exchange, gene conversion or unequal SCE is strongly stimulated in *hpr1Δ* strains; (4) *hpr1Δ* strains show a 100-fold increase in frequency of chromosome loss relative to wild-type levels; and (5) the hyper-deletion phenotype of *hpr1Δ* strains is completely absent in *rad1 rad52* double mutants but is only partially suppressed in single mutants.

Our results indicate that most of the deletions do not occur through reciprocal exchange in *hpr1Δ* strains. Spontaneous deletions between DNA repeats were previously shown to have properties different from those of other recombination events such as gene conversion. Jackson and Fink (1981) showed that gene conversion between DNA repeats was reduced over 100-fold in *rad52-1* strains, whereas deletions were reduced less than 10-fold, a result confirmed later with other DNA repeats (Klein 1989; Dornfeld and Livingston 1992). On the other hand, Schiestl et al. (1988) showed that plasmid excision occurred 100 times less frequently than deletions in wild-type cells. These results suggest that reciprocal exchange is not the only mechanism leading to deletions. We have shown in our study that deletions between DNA repeats, in wild-type strains, occur at a frequency at least 10 times higher than the frequency of reciprocal exchanges between such repeats (Table 2). These results are in general agreement with those reported by Schiestl et al. (1988). The frequency of deletions between DNA repeats in *hpr1Δ* strains was 50–200 times higher than the frequency of reciprocal exchange between such repeats. Therefore, the proportion of reciprocal exchanges responsible for deletions in *hpr1Δ* strains was less than 0.5% in the system studied (Table 3). These results are consistent with those showing a very small effect of the *hpr1Δ* mutation on reciprocal exchange of an inverted repeat, gene conversion between repeats, recombination between homologous chromosomes, recombination between a plasmid and a chromosome (Table 2) and unequal SCE (Table 4). Therefore, reciprocal exchange is not the predominant mechanism leading to deletions in *hpr1Δ* strains. However, the mechanism responsible for deletions is recombinational, because it requires the products of the recombinational genes *RAD1* and *RAD52*. This effect is partial: only the *rad1 rad52* double mutant completely eliminates the hyper-deletion phenotype of *hpr1Δ* strains. This suggests that there may be more than one recombinational pathway involved in the formation of deletions.

Our results suggest that the deletions in *hpr1Δ* strains occur predominantly through a non-conservative mechanism of recombination. A simple and attractive model to explain deletions, strongly stimulated in *hpr1Δ* strains, would be the SSA model proposed for the repair of DSB in mammals (Lin et al. 1984).

However, the event that initiates a deletion in *hpr1Δ* strains remains to be identified; and the hypothetical non-conservative recombination mechanism responsible for deletions in *hpr1Δ* strains requires the Rad52 function, in contrast to the proposed SSA mechanism for DSB repair in yeast (Ozenberger and Roeder 1991; Fishman-Lobell et al. 1992).

If the event that initiates deletions in *hpr1Δ* strains involved, directly or indirectly, a DNA break, we would expect the hyper-deletion phenotype of *hpr1Δ* strains to be accompanied by an increase in chromosome instability. Our results are consistent with this hypothesis: chromosome loss was increased 100-fold in *hpr1Δ* vs *HPR1* strains (see Table 5). Therefore we may speculate that the absence of an active Hpr1 protein in the cell facilitates the formation of DNA breaks, as a consequence of the action of nucleases, changes in chromatin structure, or alterations of DNA metabolism. The presence of DNA direct repeats at both sides of a break would allow its non-conservative repair, causing the hyper-deletion phenotype. However, if the break occurs in a chromosomal region that does not contain DNA repeats (that is, most of the yeast genome), either it is repaired by the intermolecular recombinational pathway between sister chromatids or homologous chromosomes, or it is not repaired at all, leading to the loss of the chromosome. We do not believe that the event that initiates the deletion or the loss of a chromosome occurs as a consequence of a defect in a DNA replication protein, because mutants such as *cdc2* (DNA polymerase III), *cdc17* (DNA polymerase I), or *cdc9* (DNA ligase), show an additional mutator or MMS sensitivity phenotype and elevated frequencies of recombination between homologous chromosomes (Hartwell and Smith 1985). These phenotypes have not been observed for *hpr1Δ* strains (Table 2; Aguilera and Klein 1990).

The importance of the Hpr1 protein in genome integrity may not be the same for all chromosomal regions or DNA sequences. For example, in our System 1, deletions of 14 kb occur in 2% of the *hpr1Δ* cells. If the number of events that initiate deletion or chromosome loss were equal to this value across the genome, the total number of these events would be so high as to produce deleterous consequences for *hpr1Δ* cells. This is not the case. Therefore, in the absence of Hpr1, the initiation events may not occur randomly but, rather, preferentially at particular DNA sequences or in certain chromosomal regions. Yet, the Hpr1 protein could serve to prevent initiation events from occurring across the genome (i.e., by impeding the access of a nuclease, by altering the chromatin structure, etc.), but the particular regions containing potential initiation sites would be more sensitive to the lack of Hpr1 protein. It would be very interesting to identify the molecular event that initiates the deletion in *hpr1Δ* strains. In this context, we have not been able to detect an accumulation of specific DSBs in *hpr1Δ rad50S* strains in System I (data not shown). However, this could easily be explained by a possible lack of effect of the *rad50S* mutation on the accumulation of DSBs in mitosis (Cao et al. 1990).

The Hpr1 protein may be a component of the chromatin. We have found that the analysis of the predicted amino acid sequence of the Hpr1 gene reveals the basic amino acid motif **KK**lqdaReyKig**K**e**RKK** (from amino acid 634 to 650), which fits the consensus sequence suggested for nuclear targetting by Dingwall and Laskey (1991). Also, Hpr1, together with Top1, Top2 and Top3 is required for the stability of different intrachromosomal repeats (Christman et al. 1988; Wallis et al. 1989), and *top1 hpr1*, *top2 hpr1* and *top3 hpr1* double mutants show a very reduced growth phenotype as compared with single mutants (Aguilera and Klein 1990; unpublished). Moreover, a partial sequence homology between Hpr1 and yeast Top1 and mammalian Rag1 proteins has been shown (Aguilera and Klein 1990; Wang et al. 1990) although its biological significance has not been established. Finally, it has been recently shown that the Hpr1 protein may be involved in gene expression (Fan and Klein, personal communication). Several other regulatory proteins have also been suggested to be components of the chromatin (for reviews see Grunstein 1990; Winston and Carlson 1992).

Finally, the results reported in this study invite the speculation that although DNA repeats are a potential source of genome instability, they may also play an important role in genome integrity. The integrity of a eukaryotic chromosome would depend on proteins such as Hpr1 that stabilize DNA repeats and chromosomes. However, under conditions of severe DNA damage, direct repeats become essential for the repair of resulting DNA breaks by a non-conservative recombination mechanism that causes a deletion but ensures the mitotic stability of the chromosome.

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