Biochemical and Biophysical Research Communications **270,** 112–118 (2000)
doi:10.1006/bbrc.2000.2382, available online at http://www.idealibrary.com on IDEAL®

The Effects of *RAD52* Epistasis Group Genes on Various Types of Spontaneous Mitotic Recombination in *Saccharomyces cerevisiae*

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Received February 3, 2000

The role of *RAD52* epistasis group genes on spontaneous mitotic recombination was examined using three different types of spontaneous mitotic recombination in *Saccharomyces cerevisiae.* The spontaneous recombination between homologous sequences in a plasmid and a chromosome was essentially unaffected by null mutations in any of the *RAD52* epistasis group genes. Recombination between genes in separate autonomously replicating plasmids was reduced 833-fold in a *rad52* null mutant, but only 2- to at most 20-fold in *rad50, 51, 54, 55, 57* null mutants. Recombination between tandemly repeated heteroalleles in an autonomously replicating plasmid was reduced almost 100-fold in a *rad52* null mutant, but is either unaffected or slightly increased in *rad50, 51, 54, 55, 57* null mutants. The finding that *RAD50, 51, 54, 55, 57* are dispensable or marginally involved in these spontaneous recombinations suggests further that spontaneous mitotic recombination in *S. cerevisiae* might be processed by other than *RAD52* epistasis group.

***Key Words:* RAD52 epistasis group; double-strand break repair; spontaneous mitotic recombination; plasmid–chromosome recombination; plasmid recombination.**

With rare exception, double-strand breaks (DSBs) in chromosomal DNA must be repaired for cells to survive. In yeast, *Saccharomyces cerevisiae,* repair of such broken DNA ends occurs mainly by a homology-dependent recombination process (1). The genes implicated in the homology-dependent recombinational repair of DSBs in the yeast are *RAD50, RAD51, RAD52, RAD54, RAD55* and *RAD57,* the so-called *RAD52* epistasis group (for review, see 2, 3, 4, 5). It has been reported that cells with mutations in these genes are extremely sensitive to both ionizing radiation and MMS (6, 7). They are also defective for both homothallic (8, 9) and heterothallic mating-type switching (10), which are initiated by a site-specific DSB at the *MAT* locus (11, 12).

Each of the genes has been cloned and sequenced (13, 14, 15, 16, 17, 18, 19). Among them, Rad51 bears a striking sequence resemblance to the bacterial recombination protein RecA (13, 15) and also forms characteristic filaments with single- and double-stranded DNA (20, 21). Rad55 and Rad57 proteins also have slight but recognizable homology to RecA. Rad51 protein, like RecA, catalyzes an ATP- and single-strand DNA binding protein (SSB)-dependent strand exchange reminiscent of that carried out by RecA and bacterial SSB (22). Physical interactions between Rad51 and Rad52 (23, 24, 25), between Rad51 and Rad54, between Rad51 and Rad55 and between Rad55 and Rad57 have been inferred from both genetic and biochemical studies (26, 27, 28). Recently, it has been shown that the presence of Rad52, Rad54, or Rad55 and Rad57 protein stimulated the DNA strand-exchange reaction catalyzed by Rad51 (29, 30, 31, 32, 33). Thus, these results suggest firmly that recombinational repair of DSBs is mediated by a multi-protein complex comprised of *RAD52* epistasis group gene products.

It has also been shown that some of the *RAD52* group genes, for instance *RAD50, RAD51, RAD52, RAD57,* are involved in meiotic recombination. Cells carrying the *rad52-1* mutation are defective in meiotic recombination (34, 35, 36) and *rad52-1/rad52-1* diploids fail to produce viable spores (6, 35) and result in accumulation of single-strand breaks in their chromosomes during meiosis (37). Diploid strains homozygous for *rad51* mutations are impaired in meiotic recombination and have poor sporulation efficiency and extremely low spore viability (6, 38). Also, *rad50, 52, 57* mutants have been shown to be defective in meiotic recombination using RTG (Return to Growth) experiments (34, 35).

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0006-291X/00 $35.00

In contrast to their involvement in DSB-induced recombinational repair and meiotic recombination, the role of the *RAD52* group genes in spontaneous mitotic recombination is less clear. Interestingly, it has been shown that *rad51, rad54, rad55, rad57* null mutants are considerably more proficient in recombination at intrachromosomal inverted repeats than *rad52* null mutants (39, 40, 41). In the case of intrachromosomal direct repeat recombination, it was reduced 8- to 20-fold in the absence of *RAD52* function, while the same recombination was increased in the absence of *RAD51, RAD54, RAD55, RAD57* (41, 42). The effect of *RAD50* mutation has not been known yet in these types of recombination. These results may suggest that, unlike DSB-induced recombination, spontaneous mitotic recombination might be mediated by functions other than *RAD52* group genes, especially independent of *RAD51* function. However, whether such effect is pertain to a particular recombination system or is generally applicable to other types of spontaneous mitotic recombination has not been known and remains to be determined in order to further understand the mechanism(s) of spontaneous mitotic recombination. Thus far, systematic comparisons of the effect of *RAD52* group genes have been restricted to recombination between intrachromosomal direct or inverted tandem repeats.

To address this issue, I have examined the effect of the *RAD52* group genes, including *RAD50*, in three different and previously unexamined assays of spontaneous mitotic recombination between heteroalleles in: (1) a chromosome and a plasmid, (2) two separate low-copy plasmids, and (3) between the tandemly arrayed heteroalleles in a single autonomously replicating plasmid.

MATERIALS AND METHODS

Strains. A list of strains used in this study is shown in Table 1. YME2 is a derivative of JM116 (*MATa, ura3-52, leu2--Δ1*) and S288C (43). It contains a 219 bp deletion between the *Hpa*I and *Stu*I restriction sites in the *ade*2 gene and a 187 bp deletion between adjacent *Hin*dIII sites in the *his*3 gene (44). Deletions in genes of the *RAD52* epistasis group were introduced into YME2 using the previously described method (45). The disruption plasmid for *RAD52* was obtained from David Schild. The *RAD50, RAD51, RAD54, RAD55, RAD57* disruption plasmids were provided by Rodney Rothstein. W303 isogenic strains containing comparable deletions in each member of the *RAD52* group were also provided by Rodney Rothstein.

Media, growth conditions, and reagents. Cells were grown in rich medium (YPAD) containing 40 μg/ml of adenine, in a synthetic complete medium (SDC) or in a synthetic medium (SD) containing essential purine and pyrimidine bases and amino acids (43). Generally, cells were grown at 30° either on solid medium or in liquid with shaking. To select for *NEO* positive cells, cells were plated in YPAD agar medium containing a final concentration of 500 μg of active G418/ml. Restriction enzymes were from New England BioLaboratory, G418 was from GIBCO and PEG 4000 was from EM Science Co. Unless otherwise indicated, other chemicals and reagents were from Sigma Chemical Co.

TABLE 1

S. cerevisiae Strains Used in This Study

Strain[a]	Genotype
YME2	*MATa leu2- 1, ura3-52, ade2- 1, his3- 1*
YME5	*rad52*::*LEU2* derivative of YME2
YME16	*rad51*::*LEU2* derivative of YME2
YME18	*rad54*::*LEU2* derivative of YME2
YME20	*rad55*::*LEU2* derivative of YME2
YME22	*rad57*::*LEU2* derivative of YME2
W303-1A	*MATa ade2-1 can1-100 his3-11, 15 leu2-3, 112 trp1-1 ura3-1*
J526	*rad50*::*hisG* derivative of W303
U687	*rad51*::*LEU2* derivative of W303
W839-5C	*rad52*::*TRP1* derivative of W303
U671	*rad54*::*LEU2* derivative of W303
U671	*rad55*::*LEU2* derivative of W303
U673	*rad57*::*LEU2* derivative of W303

[a] YME5, 16, 18, 20, 22 are isogenic to YME2 except where indicated. J526, U687, W839-5C, U671, U672, U673 are isogenic to W303-1A except as indicated.

Transformation. Cells grown overnight in YPAD medium at 30°C were transformed with the indicated plasmid using polyethylene glycol and lithium acetate according to the method of Schiestl and Gietz (46). When large numbers of transformations were carried out, a modification of the polyethylene glycol and lithium acetate procedure described by Firmenich and Redding (47) was used. Transformants were isolated by selecting for the particular selectable plasmid marker by growth at 30°C on either SDC or SD drop-out medium.

Plasmid constructions. The plasmids used in these experiments are diagrammed in Fig. 1. The construction of pME119, a derivative of pAF30, was described in detail previously (44). pME119 contains a defective *ade*2 gene with an insertion of a 145 bp segment containing the HO-cleavage. The two plasmids pJY100*neo*5 and pYCP33*neo*Δ3 diagrammed in Fig. 1B were constructed by fusing the two defective *neo* alleles described previously (48) to the alcohol dehydrogenase (ADH) promoter from pAAH5 (49) followed by cloning into a yeast shuttle vector pRS313 (50) and YCplac33 (51), respectively. pJY260 contains the two defective *neo* alleles above as tandem direct repeats. The plasmids and details of their construction and composition are available on request.

Determination of recombination frequencies. The recombination frequencies were calculated essentially according to the median method of Lea and Coulson (52). *RAD52* epistatic group null mutants as well as wild type yeast strains were transformed either singly or doubly with corresponding plasmid(s) depending upon the recombination system being examined (see Fig. 1). Transformed cells were isolated by growing at 30°C on selection medium (either SD-single or double drop out) for the selective marker in the plasmid(s). The isolated transformants were resuspended in ddH_2O and about 500–1000 cells were plated on the following selection medium and grown for 4 days at 30°C. SD-URA medium was used for chromosomal × plasmid recombination and for intragenic plasmid recombination. For plasmid × plasmid recombination, SD-URA-HIS medium was used. In no case were there any ADE^+ or G418-resistant cells in the 500–1000 cells plated. After cells were grown, the colonies were scraped together and resuspended in 1 ml of ddH_2O with vortexing vigorously. Cells were then serially diluted so that the OD was in the range 0.1–0.5 at 600 nm where the relationship between OD and cell concentration is linear. Cell concentration was determined using 1.0 $OD_{600\,nm} = 2.4 \times 10^7$ cells/ml. A predetermined number of cells (10^4–10^6 in those cases where the recombination frequency was high

and 10^6–10^9 where recombination was very infrequent) were then plated in duplicate on recombination test plates such as SDC-ADE for measurement of recombination between defective *ade2* genes or on G418 plates for measurement of recombination between defective *neo* genes. Cells were then incubated for 4–5 days at 30°C. At the end of the incubation, the number of colonies that appeared on the recombination test plate were counted. Only cells that had undergone recombination survived on the test plates. Judged by growth in SDC-ADE or G418, the number of recombinants was linear over the 10-fold ranges of cells plated. Recombination frequencies were determined by dividing the number of recombinant colonies obtained as above by the total number of cells plated. Each measurements were repeated several times with independently produced transformants.

Other methods. Plasmid DNAs from *E. coli* were purified using Qiagen column according to the manufacturer's protocol. Standard cloning methods and DNA manipulations were performed according to the procedures described previously (53).

RESULTS

Spontaneous plasmid × chromosome recombination. Previously, Firmenich *et al.* have shown the effect of the *RAD52* group genes on a plasmid × chromosome recombination triggered with a DSB generated by a galactose inducible HO-endonuclease (44, 54). As reported, the plasmid × chromosome recombination initiated by a DSB was found to be robust (frequency $1–2 \times 10^{-1}$) and markedly impaired in cells lacking *RAD51, RAD52, RAD54, RAD55* and *RAD57* (54), confirming that the *RAD52* epistasis group genes are required for DSB-induced recombinational repair.

To assess the role of the *RAD52* group genes on spontaneous mitotic recombination, I tested the effect of null mutations in each of the *RAD52* group genes on the same plasmid × chromosome recombination but without induced DSBs. In this system, the chromosomal *ade*2 allele contains a 219 bp deletion near the 3′-end of the coding sequence and the plasmid (pME119) contains a defective *ade*2 gene with an insertion of a 145 bp segment near 5′-end (Fig. 1A). Table 2 shows that the recombination frequency in wild type cells in this assay is $2–3 \times 10^{-7}$, almost six orders of magnitude lower than that found when a DSB initiates the recombination, suggesting that the generation of single- or DSBs in homologous DNA might be a rate-limiting step in spontaneous homologous recombination. Moreover, the recombination frequency in the absence of initiating DSB was only marginally reduced in strains lacking any one of the *RAD52* epistasis group functions (Table 2). These results suggest that, unlike DSB-induced recombinational repair, this type of spontaneous mitotic recombination is processed a pathway not requiring *RAD52* group genes.

Plasmid × plasmid recombination. Because spontaneous mitotic recombination between homologous plasmid and chromosomal alleles is independent of the *RAD52* group genes but the recombination frequency in the wild type strain is rather low, I further examined the effect of the same null mutations on recombination between homologous sequences in two separate plasmids using defective *neo* alleles (Fig. 1B). The defective *neo* alleles were employed in order to eliminate any possible complications that might be caused by using endogenous genes for the recombination assay system. In this assay, recombinational elimination of either the deletion or the insertion of the plasmid's *neo* genes yields cells that are *NEO* and, therefore, G418 resistant. Note that recombination in this assay is about 100-fold more frequent than was found for spontaneous recombination between plasmid and chromosome sequences (Tables 2 and 3). This difference may reflect the greater "accessibility" of two plasmid-borne homologues to the proteins that initiate the recombination event. By this measure, it was found that recombination was reduced by nearly three orders of magnitude in the absence of *RAD52* function while the elimination of either *RAD50, RAD51, RAD54, RAD55* or *RAD57* function reduced recombination only 10–20 fold at most (Table 3). Because recombination is considerably less impaired in the absence of *RAD50, RAD51, RAD54, RAD55* and *RAD57* than when *RAD52* function is missing, it argues for that there is an alternative *RAD52*-dependent pathway in which other members of the *RAD52* epistasis group are largely dispensable. The result also suggests further that spontaneous mitotic recombination might rely on other than *RAD52* group mediated mechanism, unlike DSB-induced recombination. The relatively small decrease seen in the absence of *RAD51, RAD54, RAD55* and *RAD57* might result from recombinational events induced by spontaneous DSBs in or near either of the plasmid-borne *neo* alleles.

Intraplasmid recombination. The involvement of the *RAD52* epistasis group functions in spontaneous mitotic recombination was also tested in an assay in which the two defective *neo* alleles were arrayed as direct tandem repeats on a single low-copy plasmid (Fig. 1C). The restoration of a *NEO* gene and a G418 resistant phenotype could occur as a result of reciprocal cross-over between homologous *neo* sequences or as a consequence of the single strand annealing mechanism following spontaneous DSBs between the *neo* alleles (55, 56, 57); both of these would yield a *NEO* gene and loss of plasmid sequences between the two *neo* alleles. Alternatively, recombination could be non-reciprocal—gene conversion—resulting in elimination of the 3′ deletion but leaving the plasmid unchanged. Using this assay, it was found that there was a nearly 100-fold reduction in recombination frequency in the absence of *RAD52* function; yet the absence of *RAD51, RAD54, RAD55* and *RAD57* function resulted in a small but reproducible increase in recombination frequency. The loss of *RAD50* function resulted in only 2-fold reduction and did not appear to be important in this type of recombination either (Table 4). This indi-

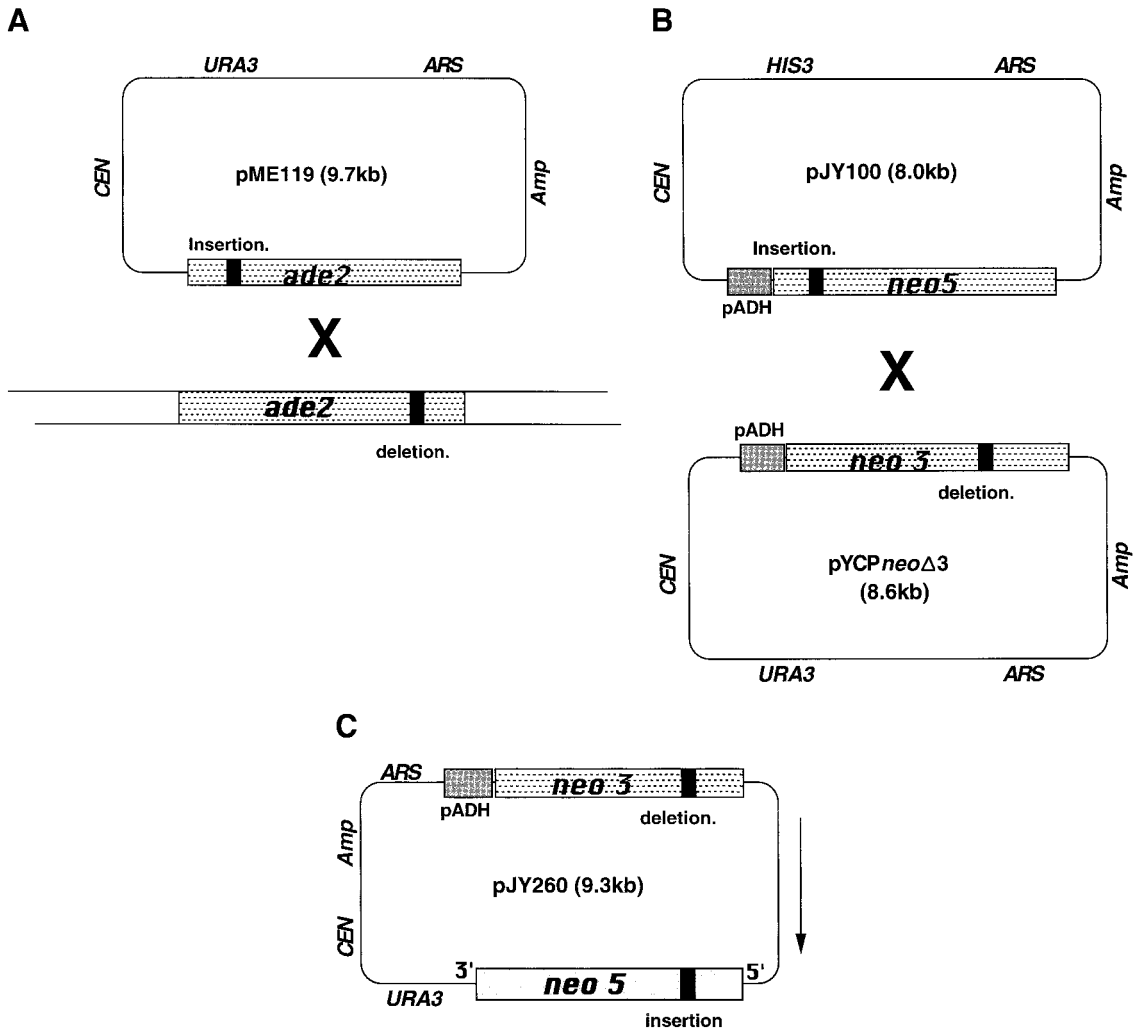

FIG. 1. Schematic representation of the recombination assay systems used in this study. Genes associated with the plasmids are indicated as follows: the ampicillin resistance gene (*Amp*), centromeric sequence (*CEN*), the yeast autonomously replicating sequence (*ARS*), *URA3* gene (*URA3*), and *HIS3* gene (*HIS3*). (A) Assay system for spontaneous recombination between plasmid and chromosomal *ade2* alleles. The *ade2* allele in pME119 contains a 145 bp insertion harboring the HO endonuclease recognition sequence near the 5′-end of the gene. The chromosomal *ade2* allele contains a 219-bp deletion near the 3′-end of the gene as described under Materials and Methods. (B) Plasmid × plasmid recombination. pJY100 contains a defective *neo* allele (*Xho*I linker insertion near the 5′-end) linked to the alcohol dehydrogenase promoter (pADH). pYCP*neo 3* contains another defective *neo* allele (173 bp deletion near 3′-end) under pADH. (C) Intraplasmid recombination. pJY260 contains two defective *neo* alleles, as shown in B, in tandem direct orientation. The *neo3* gene is driven by the ADH promoter and the *neo5* allele is promoterless. The orientation of the gene is indicated from 5′ to 3′ direction. The arrow indicates the direction of transcription.

cates that only *RAD52* among the *RAD52* group is necessary for intra-plasmid direct repeat recombination. These results further support that *RAD52*-dependent but *RAD50*, *RAD51*, *RAD54*, *RAD55*, and *RAD57*-independent recombination mechanism is responsible for spontaneous mitotic recombination.

Previously, studies of tandem direct repeat recombination has been made in chromosomal loci. McDonald and Rothstein (58) observed a small 2-fold reduction by *rad52* null strain and 3- to 4-fold hyperrecombination by *rad51*, *rad54*, *rad55*, *rad57* null strains in chromosomal direct repeat recombination using *sup4* genes containing point mutations. More recently, Aguilera (41) and Liefshitz *et al.* (42) observed 8- to 10-fold reduction by *rad52* null strain and hyperrecombination by the rest of the *RAD52* group null strains in a similar recombination assay. Compared our results to these, some differences were found between plasmid and chromosomal direct repeat recombination. First, our plasmid direct repeat recombination frequency (3×10^{-4}) is much more frequent and is up to two orders of magnitude higher than the chromosomal counterpart. Despite of such increased recombination frequency, however, similar hyperrecombination phenotype was still observed by *rad51, rad54, rad55, rad57* null strains. Second, *RAD52* function was found to be re-

TABLE 2

Effect of Null Mutations in Genes of *RAD52* Epistasis Group on Chromosomal–Plasmid Recombination without HO-Break

Strain[a]	Recombination frequency[b]	Fold decrease[c]
wt. (YME2)	2.7×10^{-7}	—
rad51	5.5×10^{-8}	5
rad52	3.7×10^{-8}	7
rad54	5.3×10^{-8}	5
rad55	6.6×10^{-8}	4
rad57	8.3×10^{-8}	3

[a] The disrupted *rad* gene in a strain otherwise isogenic to the wild-type strain. Strain names are listed in Table 1.

[b] The recombination frequency is the median value of the recombination frequencies obtained from four independent measurements determined as described under Materials and Methods.

[c] Indicates the fold-decrease in the recombination frequency compared to the wild-type strain.

TABLE 4

Effect of Null Mutations in Genes of *RAD52* Epistasis Group on Intraplasmid Direct Repeat Recombination

Strain[a]	Recombination frequency[b]	Fold increase or (decrease)[c]
wt. (W303)	3×10^{-4}	—
rad50	1.4×10^{-4}	(2)
rad51	8×10^{-4}	3↑
rad52	5×10^{-6}	(74)
rad54	8×10^{-4}	3↑
rad55	13×10^{-4}	4↑
rad57	4×10^{-4}	1↑

[a] See legend in Table 2.

[b] The recombination frequency is the median value of the recombination frequencies obtained from three independent measurements determined as described under Materials and Methods.

[c] Numbers in parentheses indicate the fold-decrease over the recombination frequency of wild-type strain, otherwise the fold-increase indicated by arrows.

quired more in plasmid recombination than chromosomal direct repeat recombination as *rad52* null strain showed a much bigger deficiency—a nearly 100-fold reduction—in plasmid recombination (Table 4), providing unequivocally a clear evidence for the differential effect of between *RAD52* and the rest of *RAD52* epistasis group genes including *RAD50* in spontaneous direct repeat recombination.

DISCUSSION

In *S. cerevisiae*, recombinational repair of DSBs during mitotic growth relies on the products of at least six genes: the so-called *RAD52* epistasis group, *RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55* and *RAD57* (4). This requirement is evident when DSBs are created by ionizing radiation (6, 59, 60, 61), in the normal course of mating type switching (8, 9, 10, 11), or in transformation following introduction of linear DNA whose ends are homologous to a chromosomal DNA sequence (61, 62). The latter cited experiments led to the suggestion that all recombination, mitotic and meiotic, is initiated by DSBs and accounts for the requirement of the *RAD52* group's encoded proteins (63, 64). However, it has been reported also that recombination between chromosomal multiple direct repeats (e.g., in rDNA (56, 65, 66) or constructs of a tandemly repeated *CUP1* gene (56) and intrachromosomal crossover of *his4* gene (67) were independent of the *RAD52* function, suggesting a *RAD52*-independent recombination mechanism. More recently, as aforementioned, chromosomal inverted repeat recombination was shown to be markedly impaired in the absence of *RAD52*, but it is only slightly reduced in the absence of *RAD51*, *RAD54*, *RAD55* and *RAD57* (39, 40, 41). Similar observations have also been made in chromosomal direct repeat recombination although the requirement of *RAD52* is much less than in chromosomal inverted repeat recombination (41, 42). These findings suggested that another alternative pathway, namely *RAD52*-dependent but *RAD51*, *RAD54*, *RAD55*, *RAD57*-independent pathway, might be responsible for spontaneous mitotic recombination. To date, however, the examination of effect of *RAD52* epistatic group genes on spontaneous mitotic recombination has been limited to chromosomal direct or inverted recombination assays. Thus, further studies of the role of the *RAD52* group in various types of spontaneous recombination substrates are warranted to better understand the mechanism(s) of spontaneous mitotic recombination.

The present study have explored the requirement for the *RAD52* epistasis group genes including *RAD50* in three different types of recombination in the absence of induced DSBs. Recombination between chromosomal

TABLE 3

Effect of Null Mutations in Genes of *RAD52* Epistasis Group on Heteroallelic Plasmid–Plasmid Recombination

Strain[a]	Recombination frequency[b]	Fold decrease[c]
wt. (W303)	2.5×10^{-5}	1
rad50	1.2×10^{-5}	2
rad51	1.8×10^{-6}	14
rad52	3.0×10^{-8}	833
rad54	1.8×10^{-6}	14
rad55	1.2×10^{-6}	20
rad57	2.4×10^{-6}	10

[a,c] See legend in Table 2.

[b] The recombination frequency is the median value of the recombination frequencies obtained from four independent measurements determined as described under Materials and Methods.

and extrachromosomal homologues is rare ($\sim 10^{-7}$) resulting in removal of the chromosomal deletion or the plasmid's insertion with equal frequency (data not shown). In contrast to the nearly 10^6-fold greater frequency of recombination between the same chromosomal and plasmid alleles initiated by a DSB in the plasmid allele, and the very substantial dependence upon the function of the *RAD52* epistasis group genes (44, 54), the frequency of spontaneous chromosome-plasmid recombination is reduced only 3- to 7-fold in null mutants of the *RAD52* epistasis group of genes (Table 2). Thus, it appears that spontaneous mitotic recombination might not be initiated by a DSB, or that initiating DSB, if it is, in spontaneous mitotic recombination are not accessible to the *RAD52* epistasis group proteins.

Further examination of another spontaneous mitotic recombination event, namely, recombinational repair of a deletion or insertion in homologues located on two simultaneously maintained plasmids (Fig. 1B), has revealed a dramatic differential effect of the *RAD52* epistasis group genes. In wild-type cells, such a recombination event occurs 100-fold more frequent than the spontaneous chromosome-plasmid recombination (Tables 2 and 3). In this assay, loss of *RAD52* function reduces the efficiency of recombination nearly 1000-fold while the elimination of *RAD50, RAD51, RAD54, RAD55* or *RAD57* function is far less, ranging from nearly negligible for the loss of *RAD50* to 10- to 20-fold for the other members of the *RAD52* group (Table 3). The result is consistent with the existence of an alternative pathway for homologous DNA recombination, which is most likely comprised of *RAD52* and other genes that remain to be identified. Moreover, assuming that plasmid $\times$ plasmid recombination is representative of homologous chromosomal recombination, the result suggest strongly that spontaneous mitotic recombination is mediated by other than the *RAD52* epistasis group genes.

I also examined the requirements for the *RAD52* epistasis group genes in recombination between two tandemly direct repeats in a replicating plasmid (Fig. 1C). Here too, recombination is reduced nearly 100-fold in the absence of the *RAD52* product, whereas it is either negligibly affected or slightly improved by elimination of the *RAD50, RAD51, RAD54, RAD55* and *RAD57* gene products (Table 4). As such big difference between *RAD52* and the rest of the *RAD52* group has never been demonstrated in similar chromosomal direct repeat assays, the result provides a firm evidence for the differential requirement of *RAD52* group genes on this type of recombination. Taken together, these results further confirm and generalize that other than *RAD52* epistasis group-mediated pathway is responsible for spontaneous mitotic recombination in *S. cerevisiae.*

A search for mutations that affect these pathways could shed light on the mechanism(s) of spontaneous homologous DNA recombination in eucaryotes. In this regard, it should be noted that the recombination frequency (3×10^{-4}) of the plasmid direct repeat assay tested in this study was up to two orders of magnitude higher than the chromosomal counterpart. Such increased recombination frequency might be exploited usefully for developing a genetic assay to isolate mutants and genes involved in alternative recombination pathways.

ACKNOWLEDGMENT

I thank Dr. Paul Berg for helpful discussions and critical reading of the manuscript.

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