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Sister chromatid-based DNA repair is mediated by *RAD54*, not by *DMC1* or *TID1*

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In the mitotic cell cycle of the yeast *Saccharomyces cerevisiae*, the sister chromatid is preferred over the homologous chromosome (non-sister chromatid) as a substrate for DNA double-strand break repair. However, no genes have yet been shown to be preferentially involved in sister chromatid-mediated repair. We developed a novel method to identify genes that are required for repair by the sister chromatid, using a haploid strain that can embark on meiosis. We show that the recombinational repair gene *RAD54* is required primarily for sister chromatid-based repair, whereas *TID1*, a yeast *RAD54* homologue, and the meiotic gene *DMC1*, are dispensable for this type of repair. Our observations suggest that the sister chromatid repair pathway, which involves *RAD54*, and the homologous chromosome repair pathway, which involves *DMC1*, can substitute for one another under some circumstances. Deletion of *RAD54* in *S.cerevisiae* results in a phenotype similar to that found in mammalian cells, namely impaired DNA repair and reduced recombination during mitotic growth, with no apparent effect on meiosis. The principal role of *RAD54* in sister chromatid-based repair may also be shared by mammalian and yeast cells.

Keywords: DNA repair/homologous recombination/ *RAD54/Saccharomyces cerevisiae*/sister chromatid

Introduction

DNA repair is an essential component of genome maintenance in living cells. DNA lesions such as double-strand breaks (DSBs) are repaired by homologous recombination (Petes *et al*., 1991; Liang *et al*., 1998). Template sequences for such repair are found either on the sister chromatid or on the homologous chromosome (non-sister chromatid). DSB repair strongly depends on recombinational repair genes (*RAD50*, *RAD51*, *RAD52*, *RAD54* and others) that are conserved from yeast to mammals (Petrini *et al*., 1997). In the yeast *Saccharomyces cerevisiae*, DSBs are naturally induced during meiosis, and repaired primarily via the homologous chromosome (Schwacha and Kleckner, 1997). In the mitotic cell cycle, the DSB repair machinery prefers the sister chromatid as a substrate for repair (Kadyk and Hartwell, 1992). Is the choice between sister chromatid and homologous chromosome in the two alternative processes genetically regulated?

Most recombinational repair genes that are required for DSB repair in mitotic cells are also required for DSB repair during meiosis (Game, 1993) and therefore are likely to play a role in homologous chromosome-based repair as well as in sister chromatid-based repair. However, a few genes are considered to function preferentially in homologous chromosome-based repair. One of these genes was recently identified and named *TID1* (or *RDH54*) (Dresser *et al*., 1997; Klein, 1997). *TID1* functions in a diploid-specific manner, promoting recombination between homologous chromosomes. Although *TID1* is expressed in the mitotic cell cycle (Klein, 1997), it seems to play only a minor role in DNA repair during vegetative growth (Klein, 1997; Shinohara *et al*., 1997). Another gene that may be specifically involved in repair based on the homologous chromosome is *DMC1*. *DMC1* was shown to be expressed only in meiosis (Bishop *et al*., 1992), and therefore may be (although not proved to be) involved strictly in recombination between homologous chromosomes. Dmc1p is structurally similar to bacterial RecA proteins. Mutant *dmc1* cells arrest in late meiosis I prophase with DSBs accumulated to abnormally high levels (Bishop *et al*., 1992). Dmc1p interacts with Tid1p in the two-hybrid assay (Dresser *et al*., 1997), suggesting that Dmc1p and Tid1p may work together as part of a complex.

DSB repair during the mitotic cell cycle was shown to depend largely on the sister chromatid as a template (Kadyk and Hartwell, 1992). However, no genes that are preferentially involved in sister chromatid-based repair have yet been described. Accumulated indirect evidence led us to consider *RAD54* as a candidate gene: mutant *rad54* cells cannot repair DSBs induced by ionizing radiation in the mitotic cell cycle (Budd and Mortimer, 1982), but appear to handle meiotic DSBs fairly well (Shinohara *et al*., 1997; this study). Hence, *RAD54* is specifically required for repair of mitotic DSBs. Our working hypothesis tested whether *RAD54* is involved primarily in sister chromatid-based repair. *RAD54* and *TID1* share significant sequence homology with one another (Dresser *et al*., 1997; Klein, 1997). Like Tid1p, Rad54p also interacts with a RecA homologue, in this case Rad51p (Jiang *et al*., 1996; Clever *et al*., 1997). Rad54p strongly stimulates Rad51p activity in homologous DNA pairing (Petukhova *et al*., 1998).

To distinguish between sister chromatid and homologous chromosome-based repair pathways, we took advantage of a unique feature of *S.cerevisiae*, namely the return-to-growth (RTG) procedure. Yeast cells that are induced to enter meiosis initiate the recombination process with a high level of DNA double-strand breakage. When switched to mitotic growth conditions (RTG), the cells complete interhomologue recombination with high meiotic frequencies, but undergo mitotic cell division and remain diploid

(Ganesan *et al*., 1958; Sherman and Roman, 1963). Recently, we have established that upon RTG there is a rapid disappearance of meiotic features (Zenvirth *et al*., 1997); the synaptonemal complex and its related structures are rapidly dismantled, and DSBs are rapidly and efficiently repaired. DSBs were shown to be repaired during RTG in the absence of a homologue in haploid cells that initiated meiosis. In diploids undergoing RTG, meiotic DSBs were mostly repaired by a homologue-dependent pathway that involved recombination. DSBs were repaired in diploid *dmc1*Δ, but not *rad51*Δ mutants, as if a recombinational repair machinery, with characteristics similar to those found in mitotic cells, came into play during RTG (Zenvirth *et al*., 1997). From these findings we concluded that at least two pathways of DSB repair can operate during RTG. One pathway involves interhomologue recombination, whereas the other involves sister chromatid recombination. By using mutants that differentially block one of these repair pathways, we exploited the power of RTG experiments to differentiate between sister chromatid and homologous chromosome-based DSB repair, in a situation where all DSBs are of the same type. RTG experiments offer an additional advantage: meiotic DSBs induced in RTG experiments are produced naturally in a controlled manner and are efficiently handled by the cell, whereas radiation and chemicals, which are commonly used to create DSBs in mitotic cells, cause other damage in addition to DNA damage.

To examine sister chromatid-based DSB repair directly we used haploid cells. In haploids, the exclusive template available for DSB repair is the sister chromatid, whereas in diploid cells DNA sequences for repair may also be found on the chromatids of the homologous chromosome. We used haploids carrying the *sir3* mutation, which enables the cells to initiate meiosis and thereby to generate a high level of DNA DSBs (Gilbertson and Stahl, 1994). Mutations in the *SIR* genes were shown not to alter the normal frequency, timing and position of meiotic DSBs, nor the kinetics of their repair (de Massy *et al*., 1994; Gilbertson and Stahl, 1994). If such meiotic cells are returned to mitotic growth conditions, they are found to be viable and produce colonies. Thus meiosis and RTG of *sir3* haploids provide us with a new tool to test the involvement of various gene products in sister chromatid-dependent DNA repair.

We show that *RAD54* is required for sister chromatid-based repair, whereas its homologue *TID1* and the meiotic gene *DMC1* are dispensable for this type of repair. Under certain circumstances *RAD54* is involved to some extent in recombinational repair by non-sister chromatids. Our data provide evidence that meiotic DSBs can be repaired by the DNA repair machinery that usually operates in mitotic cells. In diploid cells, DSBs may be repaired by either of the two pathways, the one depending on Rad54p and the other depending on Dmc1p and/or Tid1p.

Results

A novel assay for detecting sister chromatid repair

To study DSB repair by the sister chromatid, a haploid strain was used. Haploids carry only one set of chromosomes, and therefore DSBs can be repaired only by using the sister chromatid as a substrate. In order to generate, in a natural manner, a high level of chromosomal DSBs, meiosis was induced. Meiosis in haploid was achieved using a *sir3* mutation which relieves transcriptional silencing at the normally silent mating type cassettes (Rine and Herskowitz, 1987). The haploid *sir3*Δ strain (strain 3531) was induced into meiosis by transferring the culture to sporulation medium (SPM). In order to assess cell viability, samples withdrawn from SPM at various time points were returned to mitotic growth by plating aliquots on a vegetative growth medium (yeast extract/peptone/dextrose; YEPD). The number of colonies obtained from 0 h in SPM was considered as 100% cell viability, and at the following time points cell viability was calculated accordingly. To monitor the fate of DSBs upon RTG, samples were transferred after 4 h in SPM to liquid YEPD medium, and incubation was continued at 30°C. Aliquots were taken at 1 and 2 h after transfer to YEPD, and assayed for DSBs (as explained in Materials and methods). Full viability was maintained up to 8 h in the haploid *sir3*Δ strain (Figure 1A). During meiosis, DSBs were accumulated, and following a switch to mitotic growth conditions, the number of DSBs declined rapidly (Figure 1A and B). At 4 h in meiosis, 15% of the population of chromosome III molecules were broken. Considering the high level of chromosomal DSBs, the maintenance of full viability upon RTG indicates that the broken DNA was faithfully repaired rather than degraded. Since no homologous chromosome was present, the meiotic DSBs must have been repaired using the sister chromatid.

The haploid *sir3dmc1* strain (3533) served to test this methodology further. The gene *DMC1* is known to play a major role in recombination between homologous chromosomes (non-sister chromatids) in meiosis (Bishop *et al*., 1992), and therefore the haploid mutant *sir3*Δ*dmc1*Δ strain was not expected to be different from haploid *sir3*Δ*DMC1*$^+$ in the sister chromatid repair assay. Indeed, these two strains behaved similarly, namely there was no reduction in cell viability for up to 8 h (Figure 1C), and DSBs were rapidly repaired upon switching to RTG (Figure 1C and D). At 4 h in meiosis ~45% of chromosome III molecules were broken in strain 3533, and after 1 h in RTG conditions no DSBs were detected. *DMC1* is therefore not required for sister chromatid repair during RTG.

RAD54 is essential for repair by the sister chromatid, whereas TID1 is not

Although *RAD54* and *TID1* are both recombinational repair genes that share significant sequence homology with one another, they differ in several characteristics. Mutant *rad54*Δ cells are extremely sensitive to the alkylating agent methyl methane sulfonate (MMS), while *tid1*Δ mutants do not show great sensitivity. In meiosis, the diploid *rad54*Δ mutant produces viable spores at a reasonable frequency. The *tid1*Δ mutant, on the other hand, shows poor sporulation and reduced spore viability (Klein, 1997; our data not shown). The differences in behaviour between these mutants suggested to us a basic difference in DNA repair specificity between the proteins produced by these two genes. Thus, Rad54p may be involved primarily in repair by the sister chromatid, whereas Tid1p is largely involved in DNA repair by the homologous chromosome.

To determine the effect of *RAD54* and *TID1* on sister

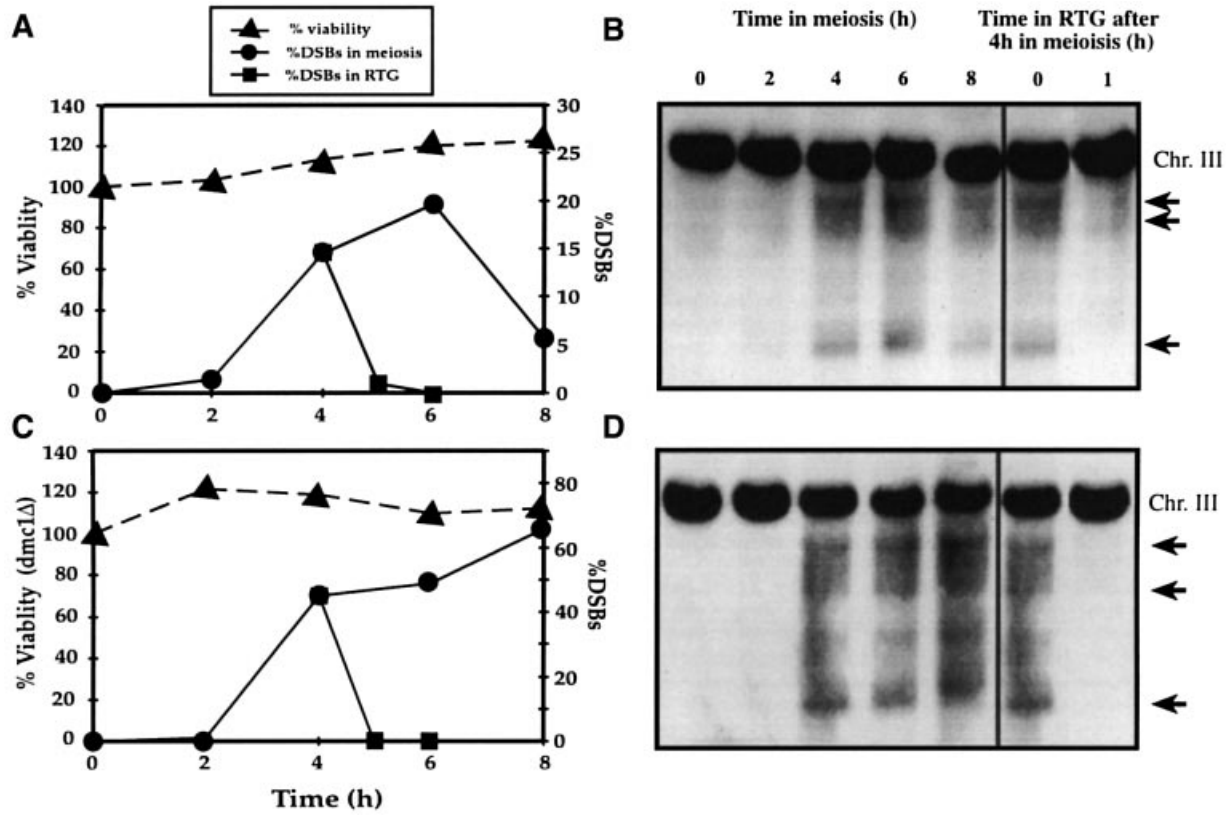

Fig. 1. Time courses in meiosis and in RTG, of DNA DSBs and cell viability, of haploid strains 3531, *sir3*Δ (**A** and **B**) and 3533, *sir3*Δ*dmc1*Δ (**C** and **D**). Aliquots of meiotic cells were taken at the times indicated, and either assayed for DSBs or returned to vegetative growth on YEPD plates and assayed for cell viability. At 4 h in the meiotic time course, a large portion of each cell culture was taken and returned to growth in liquid YEPD. Samples were taken from this RTG sub-culture at 1 and 2 h after the shift, and assayed for DSBs. (A and C) Chromosome-length DNA was electrophoresed on a CHEF apparatus, and Southern hybridized to a chromosome III-specific probe. Percentage of chromosome III breakage (%DSBs) was measured by scanning densitometry. Cell viabilities were calculated from colony counts produced by samples that were plated at various time points, relative to the number of colonies plated at time 0 h. (B and D) Representative autoradiograms, showing DSBs during meiosis, as well as at one RTG time point: 4 h in meiosis = 0 h in RTG (i.e. the shift to YEPD was made after 4 h incubation in sporulation medium). The lane of 4 h in SPM is repeated, as it represents 0 h for the RTG time course. The major DSB fragments derived from chromosome III are marked by arrows.

chromatid-based repair, we constructed a haploid *sir3*Δ-*tid1*Δ strain (3534) and a haploid *sir3*Δ*rad54*Δ strain (3532), and assayed these mutants in our sister chromatid repair assay. During meiosis of the haploid *sir3*Δ*tid1*Δ mutant, DSBs were accumulated to a high level (Figure 2B). Following a switch to mitotic growth conditions, most DSBs in cells of strain 5334 disappeared after 1 h. At 4 h in meiosis 43% of the population of chromosome III molecules were broken, and after 1 h in RTG only 9% remained broken. These residual DSBs disappeared by 2 h in RTG (Figure 2A). The DSBs disappearance was associated with high maintenance of cell viability (Figure 2A), indicating that the DSBs were faithfully repaired. These results indicate that TID1 has no essential role in sister chromatid-mediated repair. In the haploid *sir3*Δ-*rad54*Δ strain, on the other hand, although DSBs were accumulating during meiosis to a lesser extent than in the haploid *sir3*Δ*tid1*Δ, these breaks were maintained upon RTG, even 2 h after the switch (Figure 2D). The maintenance of DSBs was associated with a massive reduction in cell viability upon RTG (Figure 2C). Only 32% of the cells were viable after 6 h. These findings suggest that repair of DSBs by the sister chromatid is largely dependent on *RAD54*, and that this mode of repair does not require *TID1*.

RAD54 and DMC1 represent the two principal pathways for homologous repair of DSBs

A haploid strain deleted for the *RAD54* gene was unable to repair DSBs upon RTG. We further examined whether diploid *rad54*Δ cells are capable of DSB repair under these conditions. A diploid strain homozygous for a deletion in *RAD54* was constructed (3510). Samples were taken from this diploid *rad54*Δ sporulating culture after 0, 3.5 and 4 h and assayed for DSBs in meiosis. In addition, samples were taken from the same diploid *rad54*Δ sporulating culture after 3.5 and 4 h and returned-to-growth by transferring the cell cultures to liquid YEPD. To monitor DSBs upon RTG, samples were further taken from YEPD at 0.5 and 1 h after the switch. The amount of DSBs obtained at 3.5 h (Figure 3A) and at 4 h (data not shown) during the meiotic time course, was largely decreased after 1 h in RTG conditions. Upon 3.5 h in meiosis 18% of the population of chromosome III molecules were broken, and after 1 h in RTG only 2% of these DSBs remained. These residual DSBs disappeared completely by 2 h in RTG. The disappearance of DSBs was accompanied by a high (although not full) maintenance of cell viability (Figure 3B, compare *rad54*Δ with wild-type). *RAD54* is therefore not essential for DSB repair upon RTG in a diploid strain. This result raises the

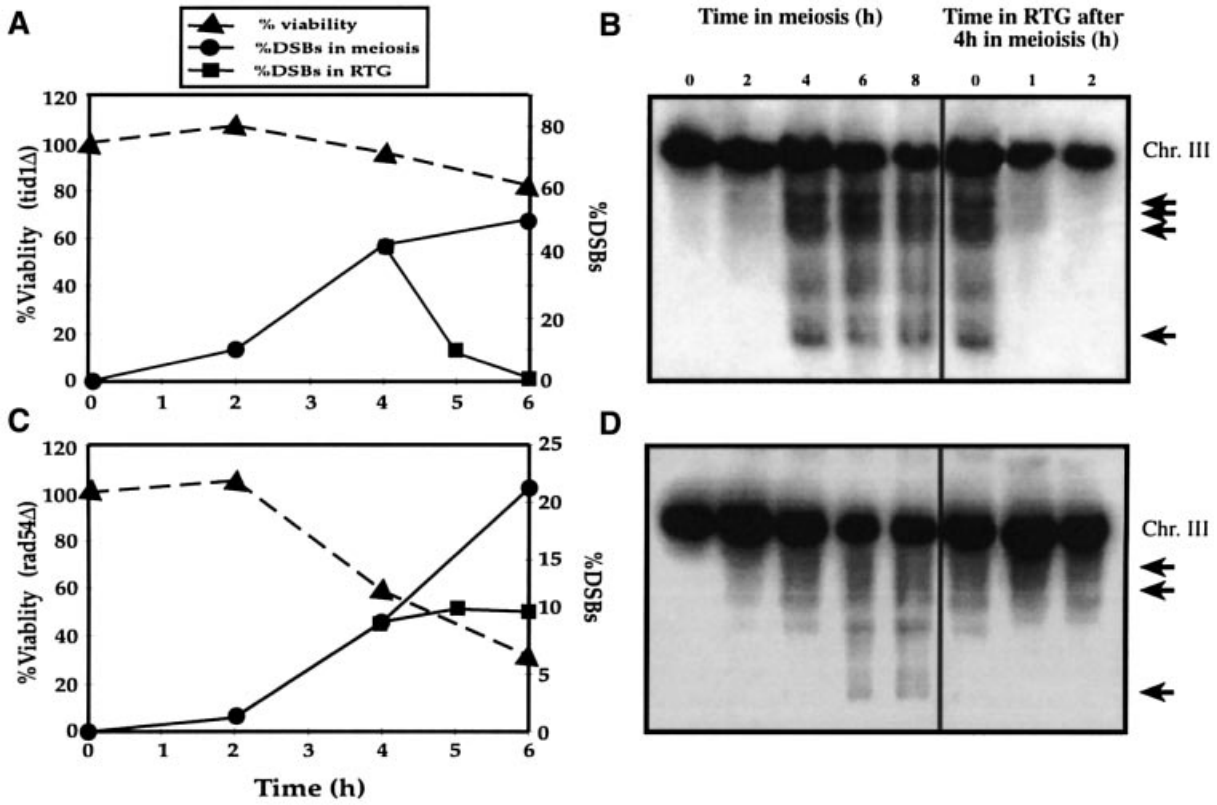

Fig. 2. Time courses in meiosis and in RTG, of DNA DSBs and cell viability, of haploid strains 3534, *sir3*Δ*tid1*Δ (**A** and **B**) and 3532, *sir3*Δ*rad54*Δ (**C** and **D**). Meiotic samples and RTG samples were taken and assayed as indicated in the legend to Figure 1. (A and C) Percentage of chromosome III breakage (%DSBs) in meiosis and in RTG and percentage of cell viability. (B and D) Representative autoradiograms, showing DSBs during meiosis, as well as two RTG time points: samples were taken from RTG 1 and 2 h after the shift to YEPD. 4 h in meiosis = 0 h in RTG (i.e. the shift to YEPD was made after 4 h incubation in sporulation medium). The lane of 4 h in SPM is repeated, as it represents 0 h for the RTG time course. The major DSB fragments derived from chromosome III are marked by arrows.

following possibility. In diploid *rad54*Δ cells, the sister chromatid-dependent repair pathway is blocked due to a deletion of the *RAD54* gene, but the non-sister chromatid of the homologous chromosome can serve as a template for repair of DSBs upon RTG. If this is indeed the case, then diploid *rad54*Δ cells are not expected to be defective in non-sister chromatid-based repair. Therefore, recombination frequencies between the homologous chromosomes in the *rad54*Δ strain are expected to be similar to those in the wild-type strain upon RTG. Recombination levels between homologous chromosomes were examined in a diploid *rad54*Δ strain (3510) and compared with a diploid wild-type strain. Both strains were heteroallelic for *his4* mutations at the *his4::LEU2* locus on chromosome III (Cao *et al*., 1990). Thus, only recombination between the two homologous chromosomes III could yield His^+ cells. Samples were taken from meiotic cultures in SPM at various time points, up to 10 h, and aliquots were plated on YEPD, as well as on defined vegetative growth medium lacking histidine. Recombination frequencies were estimated from the frequencies of His^+ colonies produced. In the wild-type strain (2982), the percentage of His^+ recombinants normally increased 200–500 fold (to 0.7–1%) during the course of the experiment (Figure 3C). In the *rad54*Δ mutant (3510), the level of His^+ recombinants was high (Figure 3C; Shinohara *et al*., 1997), and in some experiments even higher than the wild-type level. This indicates that *RAD54* is not essential for recombination between homologous chromosomes in meiosis and in RTG.

In an earlier section we showed that the *TID1* gene is not essential for sister chromatid-based DNA repair. Klein (1997) demonstrated that *TID1* is specifically required for gene conversion events that occur between homologous chromosomes. We therefore examined the ability of a diploid *tid1*Δ strain 3536 to repair meiotic DSBs upon RTG and to undergo recombination (Figure 3). In this strain the amount of DSBs obtained at 3.5 h (20%) was reduced after 1 h in RTG conditions (9%), and after 2 h in RTG no DSBs could be detected (Figure 3D). Cell viability was high, with a small reduction at 6–10 h (similar to the results obtained with the diploid *rad54*Δ strain), indicating that most of the DSBs were faithfully repaired upon RTG. In contrast to the diploid *rad54*Δ strain, the level of His^+ recombinants obtained in the *tid1*Δ strain remained very low (0.16% at 10 h; see Figure 3F). Thus, efficient repair of meiotic DSBs in the diploid *tid1*Δ strain occurs mainly by a repair mechanism that is distinct from the homologous chromosome-based repair pathway, probably by sister chromatid repair.

DMC1 was previously shown to affect recombination between homologous chromosomes (Bishop *et al*., 1992). In the *dmc1*Δ strain (NKY1879), this type of recombination is dramatically reduced upon RTG (Figure 4C, compare with the wild-type on Figure 3C). In addition, we showed that *RAD54* is required preferentially for sister chromatid-mediated repair upon RTG. Do these two genes represent the two main pathways for DSB repair? A diploid strain mutated for both *rad54*Δ and *dmc1*Δ was constructed

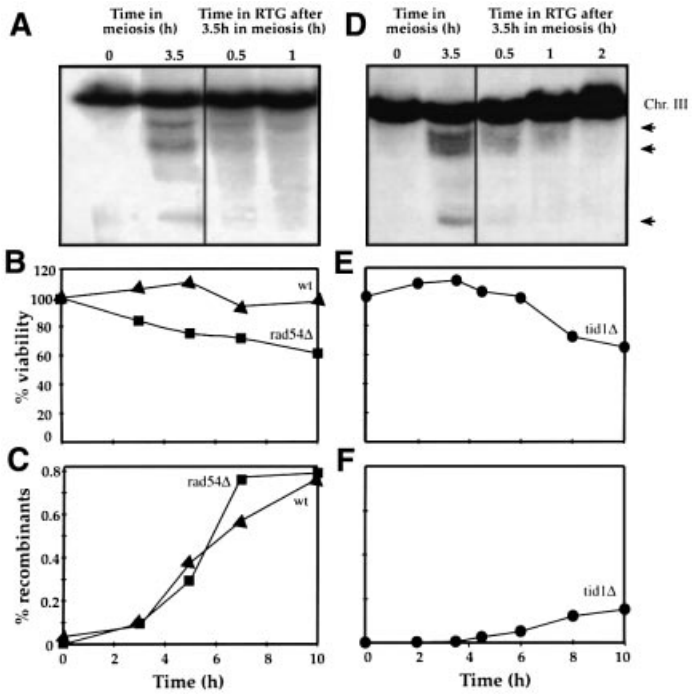

Fig. 3. DSBs, cell viability and recombination of two diploid strains: *rad54*Δ (strain 3510) and *tid1*Δ (strain 3536). Samples were taken from diploid *rad54*Δ (**A**) and from diploid *tid1*Δ (**D**) at two time-points in meiosis (0 and 3.5 h), and at three RTG time-points (0.5, 1 and 2 h after RTG at 3.5 h), and assayed for DSBs (see legend to Figure 1). The main DSBs of chromosome III are marked by arrows. (**B** and **E**) Time courses during meiosis of cell viability, and (**C** and **F**) time courses of commitment to recombination at the HIS4 locus on chromosomes III in a *rad54*Δ diploid (strain 3510), a *tid1*Δ diploid (strain 3536) and their isogenic wild-type (strain 2982). Aliquots of sporulating cells were taken at the indicated times and returned to mitotic growth either by plating the diluted culture on YEPD plates (B and E), or by plating the cells on plates lacking histidine (C and F). The frequency of recombination at HIS4 was calculated by dividing the number of His^+ colonies by the number of viable colonies on YEPD plates for each time point. Wild-type, ▲; *rad54*Δ, ■; *tid1*Δ, ●.

(3521). Cell viability and the fate of DSBs upon RTG were examined and compared with the diploid single mutants, deleted for either *dmc1* or for *rad54*. During meiosis of the diploid double mutant *rad54*Δ*dmc1*Δ, DSBs accumulated to an extremely high level (at 7 h in meiosis 73% of the chromosome III molecules were broken at least once). After the shift to RTG conditions most of the DSBs were not repaired, up to 2 h (Figure 4A). Cell viability in both *rad54*Δ and *dmc1*Δ single mutants (diploids 3510 and NKY 1879, respectively) was highly maintained upon RTG (Figures 3B and 4B, respectively). In the *rad54*Δ*dmc1*Δ double mutant, however, cell viability was severely reduced (Figure 4B). Only 13% of the cells were viable after 10 h in meiosis. These results suggests that *RAD54* and *DMC1* represent the two principal pathways for DSB repair in *S.cerevisiae*.

The *rad54*Δ*dmc1*Δ mutant was arrested in meiosis, and no spores were produced after 24 h in SPM. Meiotic arrest was previously shown for the diploid *dmc1*Δ single mutant (Bishop *et al*., 1992), whereas *rad54*Δ diploids generally complete meiosis. This indicates an epistatic effect of *DMC1* over *RAD54* in this respect.

RAD54 is also involved in some recombinational repair by the homologous chromosome (non-sister chromatid)

Recombination between homologous chromosomes in the diploid *dmc1*Δ mutant is not completely eliminated but is greatly decreased (Figure 4C; Bishop *et al*., 1992). The residual recombination events were almost abolished in a *rad54*Δ*dmc1*Δ double mutant, heteroallelic for his4 (Figure 4C). In addition, recombinant DNA molecules (which represent intermediate products in the process of recombination between homologous chromosomes) were almost absent in the *rad54*Δ*dmc1*Δ strain in meiosis (data not shown). These observations suggest that, in the absence of *DMC1*, the *RAD54* pathway may be responsible for some recombinational repair between homologous chromosomes in meiosis and possibly also in RTG.

We thus examined whether *RAD54* is involved in recombination between homologous chromosomes in the mitotic cell cycle. We used the same heteroallelic *his4* strains to examine spontaneous recombination frequencies between homologous chromosomes in mitotically dividing cells (Table I). The level of His^+ recombinants was 9-fold lower in the *rad54*Δ strain (3510) compared with the wild-type strain (2982). In similar experiments by Shinohara *et al.* (1997), a 12-fold reduction was found. Much to our surprise, a further 28-fold reduction in the level of His^+ recombinants was observed in the *rad54*Δ*dmc1*Δ strain (3521), compared with the *rad54*Δ single mutant. *DMC1* was shown to be a meiosis-specific gene and therefore its absence was not expected to further reduce the recombination level in mitotically dividing cells. As we have observed that SK1 strains of *S.cerevisiae* enter meiosis at high cell density, some of the cells in the above experiment could have embarked on meiotic recombination in YEPD. We suspected that the DMC1-dependent recombination that was observed in the mitotic culture of strain 3510 (*rad54*Δ) in fact reflected a small fraction of cells that were induced to enter meiosis. To ascertain this possibility and to ensure that no meiotic recombination

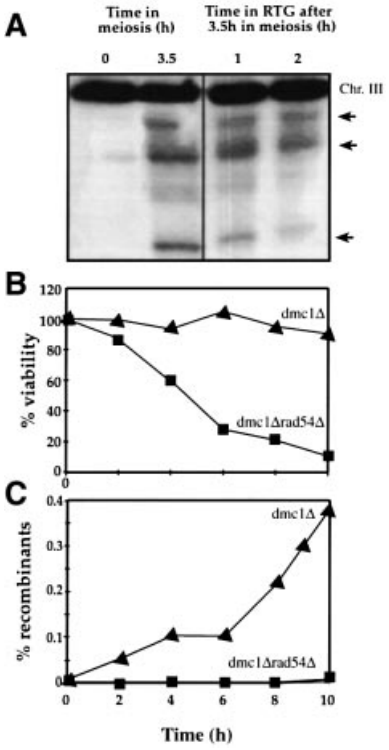

Fig. 4. The effect of deletion in both *RAD54* and *DMC1* on DNA DSBs, cell viability and recombination in diploid cells. (**A**) A representative autoradiogram showing DNA breakage at 0 and 3.5 h of the meiotic time course, in diploid *rad54*Δ*dmc1*Δ (strain 3521). At 3.5 h in meiosis an aliquot was taken and returned to mitotic growth in YEPD. Samples were further taken from this RTG subculture at 1 and 2 h after the shift. The samples were assayed for DSBs as indicated in Figure 1. The main DSBs of chromosome III are marked by arrows. (**B** and **C**) Time courses during meiosis of cell viability and commitment to recombination at HIS4 in diploid *rad54*Δ*dmc1*Δ cells (strain 3521) and in diploid *dmc1*Δ cells (strain NKY1879). Recombination frequencies and cell viability were assayed as described in Figure 3. Symbols are as follows: *dmc1*Δ, ▲; *rad54*Δ*dmc1*Δ, ■.

events would mask the spontaneous mitotic recombination, a *spo11*Δ mutation was introduced into the diploid strains. *SPO11* is a meiosis-specific gene which is required for meiotic DSB formation (Cao *et al*., 1990). *SPO11* transcripts are absent from vegetatively growing cells and the gene is transcribed specifically in early meiotic prophase (Giroux *et al*., 1989). In *spo11* mutants, spontaneous mitotic recombination occurs at a normal level and no meiotic recombination is observed (Klapholz *et al*., 1985). We used the heteroallelic *his4* site to examine spontaneous mitotic recombination frequencies between homologous chromosomes in a diploid *spo11*Δ strain (3546), a diploid *spo11*Δ*rad54*Δ strain (3543) and a diploid *spo11*Δ *rad54*Δ*dmc1*Δ strain (3551). The level of His^+ recombinants in the *spo11*Δ-deleted strain was 5-fold lower than in the wild-type strain (Table I), suggesting that the occurrence of *SPO11*-dependent recombination events (most probably meiotic events) is not negligible. In the *spo11*Δ-*rad54*Δ strain the level of His^+ recombinants was 26-fold lower than the level observed in the *spo11*Δ single mutant. Thus *RAD54* is responsible for >95% of the mitotic recombination events. However, in the *spo11*Δ*rad54*Δ-*dmc1*Δ strain the recombination level was similar to the level observed in the *spo11rad54* strain (the two values

Table I. Mitotic recombination between homologous chromosomes at the *his4* locus

Diploid strain	Abbreviated genotype	Frequency of His^+ recombinants
2982	*RAD54 DMC1* (wt)	1.59×10^{-5} (1.000)
3510	*rad54 DMC1*	1.74×10^{-6} (0.109)
3521	*rad54 dmc1*	6.15×10^{-8} (0.0035)
3546	*spo11 RAD54 DMC1*	3.04×10^{-6} (1.000)
3543	*spo11 rad54 DMC1*	1.16×10^{-7} (0.038)
3551	*spo11 rad54 dmc1*	1.02×10^{-7} (0.033)

Each value is based on at least 10 experiments.

were found not to be significantly different by *t*-test of the log-transformed values). This indicates that *DMC1* is not involved in recombination between homologous chromosomes in the mitotic cell cycle. The nature of the remaining *RAD54*-independent recombination events (occurring in $\sim1\times10^{-7}$ of the cells) remains unexplained.

Discussion

In the present study we have developed a new method based on RTG of meiotic cells containing DSBs, which enables us to identify genes that are required for sister chromatid-based repair. Our results show that the gene *RAD54* is required preferentially for DNA repair by the sister chromatid, whereas the genes *TID1* and *DMC1* are dispensable for this type of repair. The principal evidence leading to this conclusion is drawn from the behaviour of haploid *rad54* cells, which cannot repair DSBs upon RTG, because sister chromatids are the only templates available for repair. As a consequence, cell viability is severely reduced in such mutants. In contrast, haploid *tid1* and haploid *dmc1* cells showed efficient DSB repair and full viability upon RTG. We further show that *DMC1* and *RAD54* represent the two principal pathways for homologous repair of DSBs. These two pathways are able to substitute for one another in some circumstances.

RAD54 is required preferentially for sister chromatid-mediated repair

We showed that a haploid strain deleted for *RAD54* was unable to repair DSBs upon RTG, whereas diploid *rad54*Δ cells were able to repair DSBs efficiently under the same conditions. The simplest interpretation would be that *RAD54* is specifically required for repair by the sister chromatid, and in *rad54*Δ-deleted cells this type of repair is blocked. In the haploid *rad54*Δ strain, the sister chromatid, which is the only available template for repair, cannot serve for DSB reconstruction due to the *rad54*Δ mutation. Thus, a massive cell death is observed. In diploid *rad54*Δ cells, although the sister chromatid-dependent pathway cannot operate, the homologous chromosome-dependent pathway is functioning. As a result, DSBs are repaired and high viability is maintained. Indeed, in diploid *rad54*Δ cells, repair of DSBs occurs with high levels of recombination between homologous chromosomes and meiosis is almost normal. This indicates that *RAD54* is not required for DSB repair by the non-sister chromatids of the homologous chromosomes in meiosis as well as in RTG.

In normal wild-type meiosis, some events of sister

chromatid-based repair occur. The frequencies of such repair events are estimated to be between 30% (Game *et al*., 1989) and <10% (Schwacha and Kleckner, 1994) of the total DSB repair during meiosis. Interestingly, in diploid *rad54*Δ, cell viability was high, although not fully maintained. A 35% reduction in cell viability was observed after 10 h of meiosis (Figure 3B). As mentioned above, recombination between homologous chromosomes was not affected. A possible explanation of these results is that in diploid *rad54*Δ meiotic cells, a number of DSBs were committed to be repaired by the sister chromatid, and since this repair pathway was blocked, some reduction in cell viability was observed.

Other evidence supports the notion that *RAD54* is specifically required for sister chromatid-based repair. Upon RTG of the diploid *dmc1*Δ mutant, full viability was maintained (Figure 4B), as a consequence of efficient repair of meiotic DSBs (Zenvirth *et al*., 1997). The reduced level of His^+ recombinant colonies produced by this mutant, 35% of the wild-type level (Figure 4C), indicates that this repair is largely based on the sister chromatid. Indeed, in a two-dimensional gel electrophoresis assay, only inter-sister joint molecules were detected in the *dmc1*Δ mutant, upon RTG (Schwacha and Kleckner, 1997). We further suggest that *RAD54* has a major role in sister chromatid-based repair in the *dmc1*Δ mutant, since in our diploid double mutant *rad54*Δ*dmc1*Δ, a dramatic reduction in cell viability was observed, compared with *dmc1*Δ, and DSBs were not repaired upon RTG. Our interpretation of these results is that in the double mutated strain *rad54*Δ*dmc1*Δ, both principal pathways for DSBs repair are blocked (i.e. the homologous chromosome-dependent pathway and the sister chromatid-dependent pathway). Therefore, no recombinational repair of DSBs could take place.

Another gene that was proposed to have a preference for repair from the sister chromatid is *RAD50* (Ivanov *et al*., 1992). However, the ubiquitous phenotypes of *rad50*Δ mutant in DNA metabolism and chromatin structure (for representative references see Moore and Haber, 1996; Kironmai and Muniyappa, 1997) suggest a much more complex role for this gene in DNA repair.

DMC1 and TID1 are preferentially involved in repair from the non-sister chromatid and are not required in sister chromatid-based repair

DMC1 is a meiosis-specific gene that was shown to affect recombination between homologous chromosomes in meiosis (Bishop *et al*., 1992). Yet, in *dmc1*Δ mutants, this type of recombination is dramatically reduced also upon RTG (Figure 4C). It is not clear whether *DMC1* is expressed in RTG conditions, or whether the residual Dmc1p, which was induced during meiosis, persists and functions in RTG. A Northern blot analysis should be performed in order to distinguish between these two possibilities. Alternatively, *DMC1* is neither expressed nor remaining active in RTG, but its action during meiosis had committed the cell to a certain mode of recombinational repair, which is pronounced also in the following RTG conditions. In any case, the action of Dmc1p is conspicuous upon RTG.

During meiosis, the homologous chromosome (non-sister chromatid) is preferred over the sister chromatid as a substrate for DSB repair, but some level of repair by the sister chromatid does occur. Is *DMC1* involved in both types of recombinational repair during meiosis or is it specifically required in homologous chromosome-based repair? We show that *DMC1* is unable to repair DSBs using the sister chromatid, and therefore is preferentially involved in homologous chromosome-based repair. In the haploid *sir3*Δ*dmc1*Δ strain, DSBs are fully and efficiently repaired upon RTG, indicating that *DMC1* is not essential for sister chromatid-mediated repair. Moreover, in the haploid *sir3*Δ*rad54*Δ strain, the intact *DMC1* gene cannot serve to repair DSBs utilizing the sister chromatid, and as a consequence a severe reduction in cell viability is observed.

While this study was in progress, a new *RAD54* homologue was identified in *S.cerevisiae*, named *RDH54* or *TID1* (Dresser *et al*., 1997; Klein, 1997). The *TID1* gene was shown to be specifically required for recombination between homologous chromosomes. The observation that mitotic *tid1*Δ cells show no increased sensitivity to DNA damage at standard MMS concentrations has led Klein (1997) to suggest that *TID1* is not normally involved in recombinational repair by the sister chromatid. We explored this speculation and showed directly that *TID1* is indeed not required for sister chromatid-based repair. Haploid *sir3*Δ*tid1*Δ cells are able to fully repair DSBs upon RTG, and this efficient repair by the sister chromatid is translated into maintenance of high cell viability.

In the diploid *tid1*Δ strain, although recombinational repair utilizing the homologous chromosome occurred at a very low level, high cell viability was maintained during meiosis and RTG. This might be a result of the functional *RAD54* gene product that replaces the missing Tid1p during the repair of meiotic DSBs, using the sister chromatid as a substrate.

Alternative pathways for homologous repair of DNA DSBs

In the yeast *S.cerevisiae*, meiosis and the mitotic cell cycle are alternative developmental pathways. RTG conditions reflect a unique situation in which both meiotic and mitotic DSB repair pathways are largely functional (Zenvirth *et al*., 1997). Thus, RTG is a convenient tool to monitor the mutual association between these repair pathways. Upon RTG in a diploid cell, two main pathways can promote the repair of meiotically induced DSBs: the homologous chromosome repair pathway, which depends mainly on the *DMC1* gene; and the sister chromatid repair pathway, which requires the *RAD54* gene. These two pathways are able to substitute for one another, at least in part, since DSBs are efficiently repaired upon RTG in the absence of either *DMC1* (Zenvirth *et al*., 1997) or *RAD54* (Figure 3A), and as can be deduced from the high viability of cells lacking either genes upon RTG (Figures 3B and 4B). These two pathways might be able to substitute for each other also during meiosis. This conclusion is drawn from the following findings. First, DSBs that are induced in *rad54*Δ cells during meiosis (Figures 2D and 3A) disappear after 8–10 h (data not shown, and Shinohara *et al*., 1997), indicating that the main DSB repair machinery in meiosis, which is based on the homologous chromosome as a substrate for repair, does not require *RAD54*. Hence, in meiosis, the *DMC1* pathway can handle

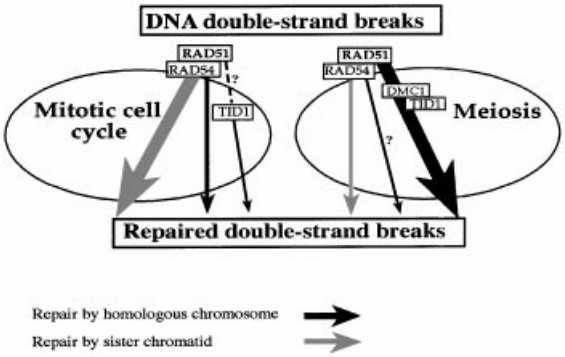

Fig. 5. Proposed pathways for DSB repair during meiosis and during the mitotic cell cycle. In meiosis, the main pathway for DSB repair is by recombination with the non-sister chromatid (in the homologous chromosome), and depends on the genes *DMC1* and *TID1*. Another possible, yet minor pathway for repair during meiosis uses the sister chromatid, and probably depends on the *RAD54* gene. In the mitotic cell cycle, the main pathway for DSB repair utilizes the sister chromatid and depends on the *RAD54* gene. However, some minor homologue-mediated repair also exists in mitotically dividing cells, which depends on *RAD54* and/or *TID1*. The major pathways are indicated as thick arrows.

most of the DSBs in the absence of *RAD54*. Secondly, the mutation *dmc1*Δ was shown to cause a meiotic cell arrest at the pachytene stage, before DSBs are repaired (Bishop *et al*., 1992; Rockmill *et al*., 1995). In search of high copy suppressors of the meiotic arrest and spore inviability phenotypes of the *dmc1*Δ mutation, *RAD54* was isolated (D.K.Bishop, personal communication), suggesting that the *RAD54* pathway may be able to repair DSBs also in meiosis. A third observation supporting this notion comes from the effect of a *red1*Δ mutation on the *dmc1*Δ arrest (Xu *et al*., 1997). A *red1*Δ*dmc1*Δ double mutant does not arrest in meiosis, and DSBs are repaired primarily by sister chromatid recombination (Schwacha and Kleckner, 1997) (the *RED1* gene appears to channel recombination repair in meiosis towards the non-sister chromatid of the homologous chromosome, and in the *red1*Δ mutant this bypass is abolished). However, in the triple mutant *red1*Δ*dmc1*Δ*rad54*Δ, DSBs remain unrepaired and there is no meiotic arrest (D.K.Bishop, personal communication); i.e. repair from the sister chromatid cannot take place.

The emerging picture is that two principal homologous recombinational DSB repair pathways exist in *S.cerevisiae* (Figure 5). One recombinational repair pathway employs the sister chromatid as a template for repair. This pathway, which involves *RAD54* and most probably *RAD51*, is the main mechanism for DSB repair during the mitotic cell cycle. (The extreme sensitivity to ionizing radiation seen in *rad54*Δ and in *rad51*Δ cells is due to their important role in sister chromatid-mediated DNA repair.) The other pathway employs the non-sister chromatid of the homologous chromosome, and involves the Dmc1p–Tid1p complex (and also Rad51p). This is the main pathway for DSB repair in meiosis. The two pathways can substitute for one another in some circumstances (as discussed above). Yet, minor repair pathways do exist both in mitosis and in meiosis. In the mitotic cell cycle, minor pathways employing the homologous chromosome are *DMC1*-independent, as our *spo11*Δ*rad54*Δ*dmc1*Δ diploid showed the same level of recombination between homologous chromosomes as the *spo11*Δ*rad54*Δ diploid (Table I). In addition, it is known that *DMC1* is not expressed in mitotic cells. One of these *DMC1*-independent pathways is *RAD54*-dependent, as we found that in the *rad54*Δ diploid strain the level of recombination between the homologous chromosomes was lower than in the wild-type strain (Table I). The other pathway is *RAD54*-independent and *TID1*-dependent (Klein, 1997). In meiosis, a minor DSB repair pathway based on the sister chromatid also exists. As was shown by the limited loss of viability of meiotic *rad54*Δ cells, *RAD54* may be involved in this DSB repair pathway. *RAD51* has been shown to be required for repair of DNA damage in the mitotic cell cycle as well as in meiosis (Shinohara *et al*., 1992), and therefore may act as a key gene in both pathways.

Testing sister chromatid-based repair

Detection of recombinational repair events that occur between non-sister chromatids is relatively easy because they differ from each other at various sites. A new linkage relationship between two or more heterozygous markers is usually analyzed. In contrast, recombinational repair between sister chromatids is difficult to detect because exchange between two identical DNA molecules does not alter linkage relationships. Our new way to evaluate sister chromatid-based DSB repair uses haploids, because in haploids the sister chromatid is the only template available for homologous DSB repair. High level of DSBs is generated upon induction of meiosis. To induce meiosis and meiotic DSBs in haploid strains, we have employed the *sir3*Δ mutation, in addition to mutations in DNA repair genes. DSBs in our method are naturally induced, and are not associated with other damage to DNA (e.g. single-strand nicks that are commonly produced by ionizing radiation) or to other cellular components. The fate of these DSBs upon RTG is assayed physically, simultaneously with cell viability. If the amount of DSBs is reduced and cell viability is maintained, the conclusion is that efficient DSB repair has occurred. If the high level of DSBs persists upon RTG and cell viability is reduced, the conclusion is that DSB repair could not have taken place. This method of testing repair of DSBs in haploids enabled us to identify genes that are preferentially involved in sister chromatid-based repair.

Several types of approaches have been previously used to detect sister chromatids recombination (Petes and Pukkila, 1995). One is the genetic detection of unequal sister chromatid exchanges between repeated genes on the chromosome (Jackson and Fink, 1985). Unequal sister chromatid exchange reflects an ectopic type of recombination and therefore might represent atypical features of recombination. The second approach involves the detection of topological changes resulting from recombination between sister chromatids of circular DNA molecules (Haber *et al*., 1984; Game *et al*., 1989). The third method is a physical analysis that detects joint molecules generated during sister chromatid recombination by two-dimension gel electrophoresis (Collins and Newlon, 1994; Schwacha and Kleckner, 1994). Our method of using RTG cultures of *sir3*Δ haploids with meiotic DSBs is an important addition to the ways in which sister chromatid repair can be assayed.

Table II. Yeast strains

Strain	Genotype	Source
2982	*MATa ho::LYS2/MATα ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG his4B::LEU2/his4X::LEU2-BamHI-URA3* (NKY1378×NKY816)	N.Kleckner
NKY1879	*MATa ho::LYS2/MATα ho::LYS2 ura3/ura3 leu2::hisG arg4-Msp/leu2::hisG/arg4-BglII dmc1::LEU2/dmc1::LEU2 his4B::LEU2/his4X::LEU2-BamHI-URA3*	N.Kleckner
3510	*MATa ho::LYS2/MATα ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG rad54::URA3/rad54::URA3 his4B::LEU2/ his4X::LEU2-BamHI-ura3*	this study
3536	*MATa ho::LYS2/MATα ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG tid1::URA3/tid1::URA3 his4B::LEU2/ his4X::LEU2-BamHI-ura3*	this study
3521	*MATa ho::LYS2/MATα ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG rad54::URA3/rad54::URA3 dmc1::LEU2/ dmc1::LEU2 his4B::LEU2/his4X::LEU2-BamHI-ura3*	this study
3531	*MATa ho::hisG lys2 ura3 leu2::hisG his4B::LEU2 trp1::hisG sir3::TRP1*	this study
3532	*MATa ho::hisG lys2 ura3 leu2::hisG his4B::LEU2 trp1::hisG sir3::TRP1 rad54::URA3*	this study
3533	*MATa ho::LYS2 lys2 ura3 leu2::hisG his4B::LEU2 trp1::hisG sir3::TRP1 dmc1::LEU2*	this study
3534	*MATa ho::hisG lys2 ura3 leu2::hisG his4B::LEU2 trp1::hisG sir3::TRP1 tid1::URA3*	this study
3546	*MATa ho::LYS2/MATα ho::LYS2 lys2/lys2 ura3/ura3 spo11::hisG-URA-hisG/spo11::hisG-URA-hisG his4B::LEU2/ his4X::LEU2-BamHI-ura3*	this study
3543	*MATa ho::LYS2/MATα ho::LYS2 lys2/lys2 ura3/ura3 spo11::hisG-URA-hisG/spo11::hisG-URA-hisG rad54::URA3/ rad54::URA3 his4B::LEU2/his4X::LEU2-BamHI-ura3*	this study
3551	*MATa ho::LYS2/MATα ho::LYS2 lys2/lys2 ura3/ura3 spo11::hisG-URA-hisG/spo11::hisG-URA-hisG rad54::URA3/ rad54::URA3 dmc1::LEU2/dmc1::LEU2 his4B::LEU2 his4X::LEU2-BamHI-ura3*	this study

The principal role of RAD54 in sister chromatid-mediated DSB repair may be shared by yeast and by vertebrates

Recently, it has been shown that repair of DSBs in mammalian cells is highly dependent on homologous sequences (Liang *et al*., 1998). *RAD51*, *RAD54* and other recombinational repair genes have been shown to be conserved through evolution from yeast to mammals (Petrini *et al*., 1997). Moreover, the human homologue of *RAD54* (*hHR54*) can substitute functionally for the *S.cerevisiae* gene (Kanaar *et al*., 1996). Disruption of *RAD54* in *S.cerevisiae* and in mouse ES cells results in a qualitatively similar phenotype, namely cells are sensitive to ionizing radiation and to MMS (Essers *et al*., 1997). *RAD54* was shown to have only a minor role in meiosis of *S.cerevisiae* (Figure 3, above, and Game, 1993). Mice with a disruption of the *RAD54* gene are fertile, indicating no essential function for *RAD54* in mammalian meiosis as well (Essers *et al*., 1997). Homologous integration is greatly reduced in mouse, chicken and fission yeast (*Schizosaccharomyces pombe*) *rad54* cells (Bezzubova *et al*., 1997; Essers *et al*., 1997; Muris *et al*., 1997) as well as in *S.cerevisiae* (see our difficulties in the construction of the haploid *sir3*Δ*rad54*Δ strains, in Materials and methods). Recent findings imply that *RAD54* is required for sister chromatid-mediated repair not only in *S.cerevisiae* but also in higher eukaryotes (Takata *et al*., 1998): chicken DT40 cells deficient in the *RAD54* gene were extremely sensitive to γ-ray irradiation in G_2, as well as in the G_1 phase of the cell cycle. In contrast, wild-type DT40 cells showed increased radiation resistance in the G_2 phase relative to the G_1 phase. At the G_1 stage of the cell cycle, DNA replication has not yet occurred. Therefore, homologous DSB repair at this stage can only use the non-sister chromatid as a template. Since the non-sister chromatid is not normally involved in DSB repair in the mitotic cell cycle, wild-type cells at G_1 phase show great sensitivity to DNA damage induced by γ-rays. At the G_2 stage of the cell cycle, the sister chromatids of each chromosome are present, and serve as the main template for DSB repair. Thus, wild-type cells show increased radiation resistance in G_2 phase. In the chicken *rad54* cell line, the sister chromatid-dependent pathway is blocked, and therefore *rad54* G_2 cells show great sensitivity to γ-ray irradiation, like *rad54* G_1 cells. These results reinforce our conclusion from the yeast haploid mutants, that *RAD54* is required for DSB repair that is mediated preferentially by sister chromatids.

We therefore propose that the role of *RAD54* in sister chromatid-mediated DSB repair in yeast described in this work implies a parallel function for its mammalian and chicken homologues. Thus, vertebrate *RAD54* may have a principal role in repair of DSBs in somatic cells through recombination with the sister chromatid.

Materials and methods

Yeast strains and media

The yeast strains used in this study are listed in Table II. All strains were constructed in SK1 genetic background. Yeast strains were maintained according to standard techniques and media were YEPD, YEPA and SPM, as described previously (Kassir and Simchen, 1991). Gene disruption in yeast was obtained by one step replacement using the electroporation technique (Becker and Guarente, 1991). All strain manipulations were verified by Southern blot analysis. We experienced great difficulties in introducing the *sir3*Δ mutation into *rad54*Δ cells. We had therefore to create a *sir3*Δ-disrupted strain first (3531), and then to introduce the *rad54* deletion, in order to create a haploid *sir3*Δ*rad54*Δ double mutant (strain 3532). Thus haploid 3532 is isogenic to strain 3531, except for the newly introduced *rad54*Δ*::URA3* deletion.

Sporulation and RTG

Sporulation was performed by a three-step procedure. Yeast were grown overnight in YEPD, resuspended and diluted in YEPA for further growth overnight, then washed and transferred to SPM. Haploid cultures were also sonicated for 10 min in a water bath prior to their transfer to SPM, in order to separate aggregated cells. At intervals, samples were spun down and resuspended in liquid YEPD medium. These were the RTG sub-cultures.

Cell viability and recombination frequencies

For assessment of cell viability and the frequency of His^+ recombinants during meiosis, 0.1 ml samples were removed from the SPM culture at various times, diluted and plated on YEPD plates and on plates with defined medium lacking histidine. The frequency of His^+ recombinant colonies was determined by dividing the number of colonies growing on the histidine-deficient plates by the number of colonies on the

complete-medium plates, YEPD, which grew after 72 h incubation at 30°C.

To determine cell viability and frequency of His^+ recombinants in mitotically growing cultures, 10 colonies from each strain were grown for 18 h in liquid YEPD medium, washed and plated at the appropriate dilutions on YEPD plates and on plates lacking histidine. Appropriate log transformations of the frequencies were made before calculating the means and standard deviations, and these were used in standard *t*-tests.

DNA manipulations

For DSB analysis, 20 ml samples of cells were washed and stored in 50 mM EDTA at 4°C, until chromosomal DNA plugs were prepared, as previously described (Gerring *et al*., 1991; Zenvirth *et al*., 1992). DNA pulsed-field gel electrophoresis was in 1% agarose and 0.5× TBE buffer (Maniatis *et al*., 1982), on a CHEF-DR^{TM}II apparatus (Bio-Rad). Pulsed-field electrophoresis conditions were: 5–35 s pulses, 200 V, 18 h. Gels were blotted onto Hybond-N nylon membranes (Amersham), which were then hybridized to ^{32}P-labeled probe according to Maniatis *et al*. (1982). Fragments of chromosome III were detected using labeled 2.2 kb *Eco*RV–*Eco*RV fragment from plasmid pSG315, as probe (Goldway *et al*., 1993), or a 1.55 kb *Xho*I–*Bgl*II fragment from the *HIS4* gene, excised from plasmid B294 (provided by G.R.Fink). The total number of DSBs was quantified by scanning densitometry.

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