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ROLE OF THE HOMOLOGOUS RECOMBINATION GENES *RAD51* and *RAD59* IN THE RESISTANCE OF *Candida albicans* TO UV LIGHT, RADIOMIMETIC AND ANTI-TUMOR COMPOUNDS AND OXIDIZING AGENTS

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Abstract

We have cloned and characterized the *RAD51* and *RAD59* orthologues of the pathogenic fungus *Candida albicans*. CaRad51 exhibited more than 50% identity with several other eukaryotes and the conserved the catalytic domain of a bacterial RecA. As compared to the parental strain, null strains of *rad51* exhibited a filamentous morphology, had a decreased grow rate and exhibited a moderate sensitivity to UV light, oxidizing agents, and compounds that cause double-strand breaks (DSB), indicating a role in DNA repair. By comparison, the *rad52* null had a higher percentage of filaments, a more severe growth defect and a greater sensitivity to DNA-damaging compounds. Null strains of *rad59* showed a UV-sensitive phenotype but behaved similarly to the parental strain in the rest of the assays. As compared to *S. cerevisiae*, *C. albicans* was much more resistant to bleomycin and the same was true for their respective homologous recombination (HR) mutants. These results indicate that, as described in *S. cerevisiae*, *RAD52* plays a more prominent role than *RAD51* in the repair of DSBs in *C. albicans* and suggest the existence of at least two Rad52-dependent HR pathways, one dependent and one independent of Rad51.

Keywords

RAD51; *RAD59*; *Candida albicans*; UV; MMS; bleomycin; camptothecin

INTRODUCTION

C. albicans is a diploid yeast that causes opportunistic infections in human. Although the genetic variability of the species is well documented, population studies indicate that the organism reproduces mainly by clonal propagation with low levels of recombination (Pujol *et*

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al., 1993; Gräser *et al*., 1996; Odds *et al*., 2007). Evidence for a parasexual cycle that involves mating followed by a process of concerted chromosome loss has been recently reported, but meiosis has not been described (Hull *et al*., 2000; Magee and Magee, 2000; Bennett and Johnson, 2003). Homologous recombination (HR) during clonal reproduction or the parasexual cycle (Forche *et al*., 2008) plays an important role in the generation of diversity since it produces recombinant alleles (intragenic recombination) or a new combination of alleles of genes located on the same chromosome (intergenic recombination). In addition to creating variability, HR plays a crucial role in the repair DNA damage in *C. albicans* (Ciudad *et al*., 2004). This is not surprising since, because of its obligate diploidy, the organism always has a homologous partner to engage in recombination.

In *S. cerevisiae*, two major mechanisms of HR have been described: double strand break repair (DSBR), which may result in gene conversion with or without a crossover event, and synthesis-dependent strand annealing (SDSA), a source of gene conversion without a crossover. HR uses proteins of the Rad52 epistasis group (Rad51, Rad52, Rad54, Rad55/57 and Rad59). Rad51 plays a central role in the homology search and catalyzes strand exchange between the recombination partners. In *S. cerevisiae*, the *RAD51*-dependent pathway of recombination is the most efficient pathway for gene conversion, and is also required for repair of most DSB in mitotic cells (Symington, 2000). In fact, Rad51 is conserved through evolution, the protein of *S. cerevisiae* having a 30% identity to the ATP-ase catalytic domain of bacterial RecA (Ogawa *et al*., 1993). In the absence of Rad51, mutagenic or non-conservative pathways, such as single strand annealing (SSA) or break-induced replication (BIR) pathways are used to repair DSBs. Two kinds of BIR have been reported, *RAD51*-dependent and *RAD51*-independent. In *S. cerevisiae*, *RAD51*-dependent BIR is an efficient process that requires Rad52, Rad55/57, Rad54, and Ku, but not Rad50 or the Rad52-paralog Rad59 (Davis and Symington, 2004). A less efficient Rad51-independent BIR requires Rad52 as well as Rad59, Tid1 and the MRX complex (Malkova *et al*., 1996; 2005). In addition, spontaneous gene conversion events with or without a crossover may also occur at low frequency, in the absence of Rad51 or Rad52, but not both proteins (Pohl *et al*., 2008; Coïc *et al*., 2008).

The Rad59 protein is about half the length of the Rad52 proteins and shows homology to the conserved amino terminal region of this family (Bai and Symington, 1996). *RAD59* was identified in a screen for mutants that significantly decrease the rate of spontaneous mitotic recombination between inverted repeats in the absence of Rad51 (Bai and Symington, 1996). In this assay *rad52* was epistatic to *rad59* and *rad51*, and *rad59* was synergistic with *rad51*. For instance, although *rad59* by itself only reduced four- to fivefold the rate of mitotic recombination, reduction reached 1000-fold in the absence of Rad51. This suggests that Rad59 functions in a *RAD51*-independent recombination pathway (Krogh and Symington, 2004). The *rad59* mutation also caused a moderate sensitivity to ionizing radiation and this defect could be suppressed by over-expression of *RAD52*, but the opposite was not true. It also decreased the efficiency of single strand annealing between direct repeats. The requirements for Rad59 in SSA increased as the length of the repeats decreased (Bai *et al*., 1999; Sugawara *et al*., 2000).

In spite of the crucial role played by HR in DNA repair, generation of diversity, and evolution of *C. albicans* (Odds *et al*., 2007), reports on the recombination mechanisms and characterization of the proteins involved in the several pathways are uncommon. We and others have previously reported the crucial role of Rad52 in DNA repair and gene targeting in *C. albicans*, as well as the effect of its deletion in the filamentation of this organism (Ciudad *et al*., 2004; Andaluz *et al*., 2006; Legrand *et al*., 2007). In the present study, we have characterized *RAD51* and *RAD59* and analyzed their role in the recovery of cells exposed to several DNA-damaging agents.

MATERIALS AND METHODS

Materials

Bleomycin (Bleocin™) was purchased from Calbiochem, camptothecin was from MBL International Corporation, MMS (methyl-methane-sulphonate) and tBOOH (tert-butyl-hydroperoxide, T-Hydro® solutions in 70% H_2O) were from Aldrich. H_2O_2 (33% w/v) was from Panreac, and menadione from Sigma Aldrich. Stocks solutions of solid compounds were prepared as indicated by the manufacturer. Bleomycin and menadione were dissolved in sterile water and camptothecin in DMSO.

Strains and culture media

The strains used in this study are shown in Table 1. They were routinely grown in YPD medium or synthetic complete (SC) medium. Uri^+ prototrophs were selected on SC plates lacking uridine. Uri^- segregants were selected on YPD plates supplemented with 5-fluoroorotic acid (5-FOA). The diploid strain BY4743 from *S. cerevisiae* and null derivatives in which both alleles of *RAD51, RAD52* and *RAD59* had been deleted were obtained from Euroscarf.

DNA extraction and analysis and cell transformation

Extraction of genomic DNA, Southern blot hybridization, and PFGE have been described before (Andaluz *et al.*, 2007; Gómez-Raja *et al.*, 2008). *C. albicans* cells were transformed by electroporation in a BTX electroporation system according to Andaluz *et al*. (2006) or using the lithium acetate method described by Walther and Wendland (2003). Chromosomal preparations and electrophoretical separations of chromosomes were performed as described (Andaluz *et al*., 2007). The region containing the *RAD51* promoter (from −539 to −7 nt) was amplified by PCR with the oligonucleotides 51BamHi-F (5'-**GATCGGATCC**AGTACCAATAACACCTTGAAGCAAAG-3') and 51SalI-R (5'-**TCGAGTCGAC**GGGTTGTCAGTATTTGTGGTGT-3'), each with the indicated restriction site (showed in bold), and the Phusion® High-Fidelity DNA Polymerase (Fynnzimes). The PCR product from strains SC5314, CAI4 was directly sequenced (Macrogen USA), whereas its counterpart from strain 1001 was cloned in vector pUC19 using NEB ligase. Five clones carrying the expected size were sequenced as above.

Gene disruption of RAD51 and RAD59

Sequential disruption of both alleles of *RAD51* was performed as described by Fonzi and Irwin (1993) using strain CAI4 (*ura3/ura3*). The disruption included about two thirds of the ORF, including the HhH and ATP-binding motifs, leaving 228 bp at the 5´-end and 224 bp at the 3 ´-end (Supplemental Fig.1A and Fig.1). To construct the disruption cassette (Supplemental Fig. 1A), the flanking regions of *CaRAD51* gene were amplified by PCR using the following primers: CaRAD51-A (5'- AGC**GAGCTC**CGATGATGATGATGATGAGG-3') and CaRAD51–B (5'– GGA**AGATCT**GTGCCTTTTAGGAGTGG–3') which amplify a 378 pb fragment corresponding to the 5'-end (see Supplemental Fig. 1A); and *CaRAD51–C* (5'–ACGC**GTCGAC**GCTCAAGTTGATGGTATGTC-3') and *CaRAD51-D* (5'–CCC**AAGCTT**GCCAGTCATTAAAGCTGTAC-3') that amplify the 3` region (374 pb). These primers contain sites for *Sac*I (A), *Bgl*II (B),*Sal*I (C) and *Hind*III (D)(shown in bold). The resulting PCR products were digested with the corresponding pairs of enzymes and cloned into plasmid pMB7 (Fonzi and Irwin, 1993) linearized with the same endonucleases to yield plasmid pJ1. Digestion of this plasmid with *Hind*III and *Sac*I released the disruption cassette that was then used sequentially disrupt both *RAD51* alleles in strain CAI4. All constructs described below were confirmed by Southern blot hybridization (Supplemental Fig. 1B) using the 4.7 kb *Hind*III- *Sac*I fragment of the disruption cassette as a probe. Two independent *RAD51/rad51*-Δ heterozygotes (JGR1 and JGR2 for Uri^+, and JGR1.1 and JGR2.1for Uri^-),

and two independent *rad51* null homozygous strains (JGR5 and JGR16 for Uri$^+$, and JGR5A and JGR16A, for Uri$^-$ strains) were constructed (Table 1).

The same approach was used to disrupt both alleles of *RAD59*. The disruption included a central 404 bp fragment (from position +166 to +570) (Supplemental Fig 1C). To construct the disruption cassette (pF1), the flanking regions of *CaRAD59* gene were amplified by PCR using the following pair of primers: IPF-1–1F (5'-AGC**GAGCTC**ATGAGAGTGTAGGTGGTGGTGAT-3'), IPF1–1R (5'–GGA**AGATCT**GTGCCATCATCATCATCATCATC-3'), and IPF1–2F (5'–ACGC**GTCGA**CGTGGGGTTACGTGATCTTTATC-3'), IPF1–2R (5'–CCC**AAGCTT**ATCAAACCCAATCAGATGCGCT.-3'). These primers contain sites for *Sac*I, *Bgl*II, *Sal*I, and *Hind*III respectively (shown in **bold**). The PCR products were digested with the corresponding pairs of enzymes and cloned into plasmid pMB7 (Fonzi and Irwin, 1993) linearized with the same endonucleases to yield plasmid p*GLB1*, from which the disruption cassette (4,8 kb) could be released by digestion with *Sac*I/*Hind*III (Supplemental Fig. 1C). This cassette was used to sequentially disrupt both alleles of *RAD59*, as described above for *RAD51*, to yield two independent heterozygotes *RAD59/rad59::hisG-URA3-hisG* (BNC1 and BNC2) and two *rad59* null *rad59::hisG/rad59::hisG-Ura3-hisG* (BNC1.1 and BNC1.2) strains (Table 1) The resulting strains were verified by Southern blot using a 986 bp *Eco*RI/*Pvu*II probe from the *RAD59* clone (Supplemental Fig. 1D, lanes 1–5).

Reintegration of RAD51 in its native locus

In order to obtain a reconstituted strain with one *RAD51* allele, we first constructed a modified pMB7 (pMBX) in which one copy of *hisG* had been eliminated by treatment with *Xba*I followed by religation. Then, a 1950 pb *Xho*I-*Spe*I fragment from pGRAD51, containing the *CaRAD51* ORF, was subcloned in pMBX cut with *Pst*I and *Xba*I to generate *pJ2* (*pMB7-RAD51-URA3-hisG*) following conversion of the complementary end into blunt ends with Klenow. This cassette was released by treatment with *Hind*III and *Sac*I (see Supplemental Fig 2A) and used to transform two *Carad51*-ΔΔ (Uri$^-$) null mutants, JGR5A and JGR16A). The transformants were selected in SC minimal medium lacking uridine. Correct integration of this cassette was verified by Southern analysis using the 1059pb *Xho*I-*Eco*RI fragment of p*GRAD51* as a probe (Supplemental Fig. 2B). Only four transformants, two from each strain, could be selected on SC minimal medium (minus uridine), suggesting that the lack of Rad51 significantly impairs the efficiency of integration. However, only one derived from JGR16A (Supplemental Fig. 2B, lane 3) had regained the wild type smooth colony morphology (see results) and yielded the expected pattern when analyzed in Southern blot, i.e. it had retained one of the *rad51::hisG* alleles whereas the other had reintegrated the construct to yield the *rad51::RAD51-URA3-hisG* allele (JGR25).

Construction of a conditional strain

To generate the *CaMET3-RAD51* cassette, a 421 bp fragment, comprising positions –97 to +324 bp in relation to the first nucleotide of the *RAD51* open reading frame was amplified by PCR using the appropriate oligonucleotides MET-RAD51-F (5'-CGC**GGATCC**CCAATAATCCATTCGCGTCC-3') which includes a restriction site for *Bam*H1, and *MET-RAD51*-R (5'- AA**CTGCAG**TTCACTGGCAGTGGTGAAAC-3´) which includes a restriction site for *Pst*I (bold letters). The PCR product was cloned in plasmid *MET3-URA3* (pCaDis)(Care *et al*., 1999) following digestion both enzymes (*Bam*HI and *Pst*I) which are also present in the polylinker of the vector. The construct was digested with *Btr*I and used to transform the heterozygous *RAD51/rad51* Ura$^-$ strains JGR1.1 and JGR2.1. Transformants carrying a conditional allele were analyzed by PCR using oligonucleotides MET1, 5´-CGACATAGATCCATTAATGCGCC-3´, located in the *MET3* promotor, and MR51-R (5'-GCTAACGTGTGGCACAATTG-3'; complementary to the region + 453 − + 473 of the

RAD51 ORF) which amplify a 1.0 kb fragment (Supplemental Fig. 2C) as well as oligonucleotides MET1 and CaRAD51D which amplify a 1.8 kb fragment. Confirmed clones were then analyzed for the null allele by PCR, using primers flanking the deleted region of *CaRAD51* (CaRAD51-A and CaRAD51-D, see above) which amplify a 2.0 kb fragment (Supplemental Fig. 2D). The conditional strain was routinely grown in SC medium lacking methionine (Met), cysteine (Cys), and uridine (Uri). To repress *RAD51* expression, agar plates were supplemented with 2.5 mM Met and 2.5 mM Cys. Similarly, liquid cultures were supplemented with 2.5 mM Met and 2.5 mM Cys and inoculated with exponentially growing cells (OD_{600} lower than 2) to reach an initial OD_{600} of about 0.01.

Sensitivity to DNA-damaging agents

Strains were grown overnight in liquid YPD medium and then diluted to an $OD_{600} = 1$. Two parallel assays were carried out. In the first one, about 250–300 colony forming units (CFU) were plated on YPD. In the second assay, 5 µl of the initial cell suspension and serial fivefold dilutions were spotted onto YEPD plates. For UV treatment, cells were irradiated at the indicated doses. For the DNA-damaging compounds (MMS, camptothecin, bleomycin, and oxidizing agents) sensitivity tests, the plates had been supplemented with the indicated amounts of each compound. All plates were sealed, wrapped in aluminum foil and incubated in the dark at 28°C for the indicated periods. Then, colonies were counted (assay 1) or plates were photographed (assay 2). All the assays were repeated three to four times. Colonies from CAF2, heterozygotes, and *rad59* null strains were counted after 24 h, whereas those from *rad51* null and *rad52* null strains were counted after 36–48 and 72 h respectively.

RESULTS

Comparative analysis of CaRad51p and CaRad59p

The sequence of *RAD51* ORF (1086 bp) was first obtained from the Pasteur Institute data base (http://genolist.pasteur.fr/CandidaDB). The deduced protein (361 amino acids) showed a substantial homology to Rad51 proteins from other eukaryotes (62–68% identity), including *S. cerevisiae*, *Kluyveromyces lactis*, *Drosophila melanogaster*, and *Homo sapiens* (Fig.1), as well as to Rad51p from the *Archaea Pyrococcus furiosus*. When CaRad51 was compared to RecA from *Escherichia coli*, identity was much lower (11%) and homology was limited to a region comprising residues 37 to 272 in RecA and 113 to 352 in CaRad51. Within this region, CaRad51p carries two highly conserved domains involved in binding and hydrolysis of ATP, known as Walker A or phosphate-binding (GEFRTGKS, positions 144–151) and Walker B (FSLLIVDS, positions 231–239) motifs (Aboussekhra *et al*., 1992;Basile *et al*., 1992;Shinohara *et al*., 1992;Koonin, 1993;Thayer *et al*., 1995;Shin *et al*., 2003). CaRad51 also carries a modified HhH motif involved in non-specific binding to DNA (positions 77–86) preceded by a N-terminal domain (positions 1–76) characteristic of eukaryotic homologues (1–117 in *S. cerevisiae* and 1–59 in *H. sapiens*). Both regions are absent in RecA, which uses instead its unique C-terminal domain to bind double-stranded DNA (Kurumizaka *et al*., 1999). This C-terminal domain is absent in eukaryotic Rad51 proteins (Fig.1). Finally, CaRad51p also displayed a significant homology to Dmc1 (Fig. 1), a protein involved in meiotic recombination in *S. cerevisiae* (Bishop *et al*., 1992;Symington, 2002) whose homologue in *C. albicans*, Dlh1, has been partially characterized (Diener and Fink, 1996).

CaRad59p (Candida database; 215 amino acids) showed similarity with orthologs of other fungi including *Debaryomyces hansenii* ($2e^{-36}$), *Kluyveromyces lactis* ($2.3e^{-19}$), *Ashbya gossypii* ($2.4e^{-15}$), *C. glabrata* ($2.4e^{-15}$), and *S. cerevisiae* ($4e^{-15}$), but it was lower (26–39%) than that found for Rad51 proteins (Supplemental Table 1). In addition, the region of CaRad59 comprising residues 57 to 214 showed a 29% identity and 39% similarity with region 54–220 of CaRad52.

Cloning and restriction analysis of CaRAD51 and CaRAD59

According to the *Candida albicans* information database (http://alces.med.umn.edu/bin/fosmidlist?PRAD51+-/), three fosmids of chromosome R, 2H6, 14E6 and 15F5, were selected as candidates to carry the entire *RAD51* ORF. These fosmids were kindly provided by B.B. Magee. They contain DNA from strain 1161, a derivative of strain 1006, and have been used for the construction of the physical map of *C. albicans* (Chu *et al*., 1993). Each fosmid was digested with *Xho*I/*Hin*dIII, since these enzymes should release a fragment of about 3 kb comprising the whole *RAD51* ORF, including its promotor (774 bp) and terminator (1166 bp). This was more easily accomplished with fosmid 15F5 which was then used as a source of the gene. This fragment was cloned in vector pGEM-7Z(+) restricted with with *Xho*I and *Hin*dIII to yield pGRAD51. As expected from the information present at CGD (Candida Genome Database http://www.candidagenome.org), *RAD51* was located on chromosome R (ChrR) by Southern blot analysis of the electrophoretic karyotypes of the strain CAI-4 (not shown). A restriction analysis of the cloned *RAD51* ORF indicated the absence of one site for *Xba*I and two sites for *Sac*I, which are present in the sequence of strain SC5314 (Supplemental Fig. 1A). Since the fosmids used for cloning are derived from strain 1161, a 1006 derivative, this observation suggested the existence of RFLP in the *RAD51* locus of *C. albicans*. To further determine if this polymorphism extended to other laboratory strains of *C. albicans*, genomic DNA from strains 1001, 1006, 3153A, 4918, SC5314 and its derivative CAI4, was cut with both enzymes (*Xba*I and *Sac*I), and subjected to Southern blot (Supplemental Fig. 3A). As expected from our data with the cloned gene, parental strain 1006 lacks sites for both enzymes in both alleles, and the same was true of strain 4918. Strains 3153A, SC5314 and its CAI4 derivative carry the *Xba*I site in both alleles, whereas strain 1001 was heterozygous for this site. Strains 1001, SC5314 and CAI4 were also heterozygous for the second *Sac*I site, which corresponds to a SNP (t/g) located 172 bp upstream of the initiation codon of *RAD51* in the *Candida* database (Forche *et al*., 2004). However, the presence of the first *Sac*I site in one or both alleles of strains 1001, SC5314 and CAI4 could not be unambiguously confirmed using Southern blot. Sequencing of the promotor of the three strains (Supplemental Fig. 3B) confirmed the above results (i.e. the three strains were heterozygous for the second *Sac*I site) and indicated that the first *Sac*I site was absent in both alleles of strain 1001 and present in both alleles of strain SC5314 and CAI4. A summary of these results is shown in Supplemental Fig. 3C.

Similarly, three fosmids of chromosome 4, 28F2, 8F2, 19G5, carry *RAD59*. They were derived from strain 1161 and were also kindly provided by B.B. Magee. When digested with *Nsi*I and *Hin*dIII, all three fosmids yield a fragment of 1881pb, as predicted by the Candida database. The one derived from fosmid 8F2 was cloned in the polylinker of vector pGEM-7Z (+) restricted with the same enzymes. The construct (pGRAD59) carried the Ca*RAD59* ORF preceded by 955 bp and followed by 276 bp. A restriction map of the cloned fragment indicated the same sites predicted by the SC5314 sequence (Supplemental Fig. 1C), as well as an additional *Xba*I site in the promotor, suggesting a polymorphism among the strains. As expected, a Southern blot analysis of the electrophoretic karyotypes of SC5314 and CAI-4 derivative, using a 299bp internal probe of *RAD59*, localized the gene on chromosome 4 (not shown).

Colony and cellular morphology, growth rate and karyotypes of rad51 and rad59 null homozygotes

When grown on agar plates containing either YPD or minimal medium (Figure 2, first and second columns), both *rad51* null strains (only JGR5 is shown, fourth row) formed colonies containing both yeast and filamentous cells. Filamentous cells also formed in liquid media (Fig. 2, third column). Reintegration of the wild type allele restored the wild type morphology of both colonies and cells (Fig.2, fifth row). In agreement with these results, the conditional

strain grew as a yeast in the absence of the repressor (Cys/Met)(Fig. 2, seventh row), whereas in its presence, the strain formed filaments (Fig. 2, sixth row). However, filamentation did not reach the level of the null strain suggesting that the promotor was not fully repressed. These results suggest that, as shown for *rad52*, filamentation of *rad51* cells is not due to secondary mutations, but is due to the absence of the Rad51p. However, the filamentous phenotype was significantly less prominent in *rad51* than in *rad52* cells (Fig. 2, compare third and fourth rows). It should be noticed, that the colonial morphology of our *rad51* and *rad52* strains is unrelated to the white-opaque switching which has been shown to occur recently in "fuzzy" colonies of *MTL*-homozygous *rad52*-ΔΔ strains (Alby and Bennet, 2009), since the strains used here are *MTL*-heterozygous.

Both *rad51*-ΔΔ mutants exhibited a slight decrease in growth rate in YPD (g -generation time − = 75 min) as compared to wild type (g = 60 min), but still grew significantly faster than its *rad52*-ΔΔ counterpart (g =120 min). Reintegration of *RAD51* restored the growth rate to the level of the wild type. Both *rad59*-ΔΔ mutants (BNC1.1 and BCN2.1) showed the same generation time as the parental strain CAF2. Cellular and colonial morphologies were also quite similar to those of CAF2 (Fig. 2, second row).

The electrophoretical karyotypes of both *rad51* and *rad59* null strains were quite similar to those of parental CAI4, except for differences in the size of ChrR. However, we have shown that variation in the sizes of the ChrR homologs occurs following gene disruption or passages through 5-fluoroorotic acid (5-FOA) plates (Andaluz *et al*., 2002). When subjected to passages on YPD plates, one *rad59* null strain showed an apparently stable ChrR, whereas the other showed changes in the sizes of both homologs. For *rad51* nulls, one strain displayed a progressive increase and the other a progressive reduction in the size of ChrR (Supplemental Fig. 4).

The role of Rad51 and Rad59 in the repair of DNA lesions caused by DNA-damaging agents

Next, the sensitivity of *rad51* and *rad59* mutants towards several mutagenic and oxidizing agents was investigated. Although we will only show the results obtained for one mutant of each class, both *RAD51* heterozygotes (JGR1 and JGR2) behaved similarly in all the assays described below, and the same was true for both *rad51* null homozygotes (JGR5 and JGR16), both *RAD59* heterozygotes (BNC1 and BNC2), and both *rad59* null homozygtes (BNC1.1 and BNC2.1).

a) UV light

The main mechanism responsible for the repair in the dark of UV-induced lesions, including cyclobutane pyrimidine dimers and (6–4) photoproducts, is the nucleotide excision repair (NER) pathway (Birrell *et al*., 2001). *S. cerevisiae* NER mutants are less sensitive to UV light than *C. albicans* NER mutants (*rad2* and *rad10*) and recover more readily from UV-damage, suggesting that *S. cerevisiae* may have evolved extra pathways for the recovery of the UV damage, whereas *C. albicans* relies more on the NER pathway (Legrand *et al*., 2008). One of these pathways includes a photolyase, which seems to be absent in *C. albicans* (Miller and Sarachek 1974; Ciudad *et al*., 2004; Legrand *et al*, 2008). However, HR has been also involved in the recovery of UV-lesions, although its contribution varies among yeasts (Birrell *et al*., 2001; Jang *et al*., 1995). More specifically, Rad51 is involved in transcription-coupled repair of UV damage when homologous molecules are present (Aboussekhra and Al-Sharif, 2005), and Rad51 and Rad52 are required for post-replication repair of UV- damaged DNA in *S. cerevisiae* (Gangavarapu *et al*., 2007). Previously, we have reported that *rad52* null strains of *C. albicans* are significantly more sensitive to UV light irradiation than *lig4* null counterparts, suggesting that some of the lesions caused by this agent are repaired by HR, the NHEJ pathway playing a minor repair role at the doses used (Ciudad *et al*., 2004). In order to test whether

Rad51 or Rad59 participate in the recovery of UV lesions, exponentially growing cells from CAF2 as well as heterozygous and null homozygous strains of *RAD51, RAD59* and *RAD52* were subjected to several doses of UV light (10–30 Jm^{-2}) and survival (CFU) determined (Fig. 3). Disruption of one copy of either *RAD51* (JGR1) or *RAD52* (TCR1) caused a significant increase in UV sensitivity, both heterozygotes displaying about the same sensitivity. The *RAD59* heterozygote was also affected but to a lesser extent at all the intensities tested. Haploinsufficieny of *RAD51, RAD52*, and *RAD59* was more evident as the UV-light dosage increased, suggesting that the amount of each recombination protein, in particular Rad51 and Rad52, is a limiting factor for the recovery of UV-lesions. Both *rad51* and *rad52* null strains displayed increased sensitivities to UV light as compared to their respective heterozygous parental, but *rad52* was slight to moderately more sensitive than *rad51; rad59*-ΔΔ was also more sensitive than the heterozygote although significantly less sensitive than the *rad51* counterpart at all the UV doses tested (Fig. 3). As expected, a *RAD51*-reintegrant strain was similar in sensitivity to the heterozygous strain, a behavior previously described for a *RAD52*-reintegrant strain (Ciudad *et al*. 2004). Analysis of UV light sensitivity using the serial dilution assay confirmed that *rad52*-ΔΔ is moderately more sensitive to UV light irradiation than *rad51*-ΔΔ (Fig. 3B). In this assay, the *rad59* null strain was again more sensitive to UV exposure than CAF2 (Fig. 3C) but less than *rad51*-ΔΔ (not shown). These results confirm that HR plays a significant role in the recovery of UV-damage in *C. albicans*; furthermore, the rather small difference in sensitivity to UV light between *rad51* and *rad52* indicates that the HR-dependent recovery preferentially uses the *RAD51*-dependent pathway.

C. albicans displayed about the same UV sensitivity as a wild type diploid of *S. cerevisiae* and the same was true for the *Carad51*, and *Carad59* nulls and their *S. cerevisiae* counterparts, whereas *rad52* from *C. albicans* was more compromised than its counterpart from *S. cerevisiae* when compared to their respective controls (Fig. 3, B and C). Therefore, the role of HR in the recovery of UV-induced lesions in *C. albicans* is apparently similar or slightly more important than that reported for *S. cerevisiae* where the *RAD52* epistasis group of genes displays a modest increase in UV sensitivity (Aboussekhra *et al*., 1992; our own results). When incubation was prolonged from two to four days, a significant recovery of each *rad51*-ΔΔ and *rad52*-ΔΔ cells from either yeast was observed, an indication that other pathways are actively involved in the repair of the lesions. As noted above, it is likely that the NER pathway plays a crucial role in both the recovery of damaged cells and the residual resistance observed in *rad51* and *rad52* mutants of *C. albicans* when treated with high doses of UV light

b) Radiomimetic compounds: MMS

MMS functions as an alkylating agent that causes N-methyl adducts namely N7-methylguanine and N3-methyl adenine, the latter being cytotoxic due to blockage of replication fork progression. Although BER is the major pathway to repair these lesions, it is also known that MMS-lesions induce HR and HR mutants are sensitive to MMS, suggesting that some damage is processed to DSBs (Wyatt and Pittman, 2006). In the CFU assay (Fig. 4A), the heterozygotes *RAD52/rad52* and *RAD51/rad51* displayed slight sensitivity to MMS (0.001 to 0.01%). Both null strains showed increased sensitivity to MMS, but the *rad52* null was significantly more sensitive than the *rad51* counterpart. The *RAD51*-reintegrant strain behaved as the corresponding heterozygote. In the same assay, the *rad59* null strain behaved like wild type. The higher sensitivity of the *rad52* null as compared to the *rad51* counterpart was confirmed by using the serial dilution assay (Fig. 4B), suggesting that a Rad51-independent Rad52-dependent pathway of HR participates in survival to MMS. At first, this pathway seems to be crucial for the recovery of MMS-induced lesions, since almost no recovery was observed in the absence of Rad52 upon prolonged incubation, whereas significant recovery was observed in the absence of Rad51 (Fig. 4B). However, recovery of cells with time on MMS plates should be regarded cautiously because of the rapid breakdown of this compound in media after

exposure to light and air. In fact, when MMS plates were aged for 2, 4 and 6 days prior to the addition of cells, growth of *rad51*, and to a lesser extent *rad52*, increased in parallel, even thought the plates were permanently maintained in the dark (Fig. 4C). In the serial dilution assay, the *rad59* null was not affected by MMS concentrations ≤ 0.0015%. At concentrations of MMS (0.01% and 0.02%) that do not allow any growth of *rad51*-ΔΔ (Supplemental Fig. 5) or *rad52*-ΔΔ (not shown) strains, the *rad59* null was significantly more sensitive than the parental strain CAF2, suggesting that in the presence of Rad51 and Rad52, Rad59 facilitates repair of DSBs when the number of lesions exceeds a threshold. Again, the recovery of cells with the incubation time was prolonged was probably due to breakdown of MMS.

A wild type diploid strain of *S. cerevisiae* showed about the same (or even increased) sensitivity as the CAF2 strain of *C. albicans* in presence of 0.005% MMS, but the *C. albicans rad51* and *rad52* nulls were significantly more sensitive than *S. cerevisiae rad51* and *rad52* nulls respectively (Fig. 4C), a phenotype that was more clearly observed after 4–6 days of incubation. As mentioned above, the recovery of mutant cells observed upon prolonged incubation times was due largely to MMS breakdown.

c) Antitumor drugs: bleomycin and camptothecin

Bleomycin produces oxidative AP sites and single strand breaks (SSB) terminated by 3´-phospho-glycolate (3´-PG), but further attack to a site in the opposite band, particularly at the CGCC sequence tracks, generates DSBs. Bleomycin is highly effective *in vitro* as an antifungal agent, although for reasons that remain unclear is ineffective *in vivo* against *C. albicans* (Graybill *et al*., 1996). As shown above for MMS, in both assays, CFU and serial dilutions, *rad52*-ΔΔ was significantly more sensitive to bleomycin than *rad51*-ΔΔ, whereas the *rad59* null strain was less affected (Fig. 5A and C). In the CFU assay, *RAD51* and *RAD52* showed slight to moderate haploinsufficiency since their respective heterozygotes and reintegrants yielded lower counts than CAF2 (Fig. 5A). Both *rad51*-ΔΔ and *rad52*-ΔΔ cells showed some recovery when incubation was prolonged (Fig. 5C). Interestingly, whereas the continuous presence of bleomycin makes survival more dependent on Rad52 than on Rad51, when bleomycin treated cells were allowed to recover in the absence of the drug (Fig. 5B) both mutants, *rad51* and *rad52*, showed a similar recovery. These results suggest that in the presence of bleomycin a Rad51-independent Rad52-dependent pathway of HR (for instance, Rad51-independent BIR) contributes significantly to the recovery of the lesions, whereas, in the absence of bleomycin, all HR repair seems to use the *RAD51*-dependent pathway of HR. The residual survival of *rad52* cells in the presence or in the absence of the drug (Fig. 5A and B) as well as the partial recovery of *rad51* and *rad52* cells when incubations were prolonged in the presence of bleomycin (Fig. 5C) also suggest the existence of Rad52-independent pathways involved in the repair of the bleomycin-induced DNA lesions. Among these, BER, NHEJ and the Rad6-dependent post-replication repair pathways are likely candidates, but their contributions remain to be determined.

As shown in Fig 5C, *C. albicans* was resistant to bleomycin concentrations (2.5 and 5 μg/ml) that killed most cells of *S. cerevisiae*, and, as expected, this resistance was largely dependent on the presence of Rad52 whereas, by comparison, the contribution of Rad51 was less. The *rad59* null of *C. albicans* behaved as the parental CAF2, whereas the *rad59* null of *S. cerevisiae* was more sensitive than its parent. Several possibilities may account for the differences in bleomycin susceptibility between the two organisms. First, *C. albicans* could secrete a bleomycin-inactivating activity. To test this possibility, we incubated either live or heat-killed *C. albicans* cells with bleomycin. After 2 h, cells were centrifuged and supernatants removed and tested against *S. cerevisiae* cells in a CFU assay. No differences were found between the supernatants from viable or dead cells, arguing against an enzymatic inactivation of the drug by *C. albicans* (data not shown). Second, *C. albicans* genome could encode a potent

cytoplasmic bleomycin hydrolase activity that strongly decreases bleomycin concentration in the cell. In *S. cerevisiae*, this activity is encoded by *BLH1/LAP3* for which an uncharacterized ortholog (*LAP3*) has been identified in the *C. albicans* genome (CGD). Third, *C. albicans* could have more efficient efflux pumps involved in the efflux of bleomycin than *S. cerevisiae*. And, fourth, specific features of the *C. albicans* cell wall could limit bleomycin entry, since *S. cerevisiae* mutants lacking cell wall functions are hypersensitive to bleomycin (Leduc *et al*., 2003). In any case, there is no doubt that bleomycin is causing lesions, likely DSB, in the *C. albicans* DNA, since analysis of the karyotypes indicated chromosome degradation in the presence of bleomycin (Fig. 6). As expected from the sensitivity of several strains to the drug, chromosomal degradation was extensive for *rad51* and *rad52* null strains, and less pronounced in the *rad59* counterpart which behaved like parental CAF2. Interestingly, survivors from bleomycin treatment that were able to form colonies on YPD plates in the absence of the drug had regained the karyotype profile typical of the untreated strain. This observation was true for wild type or each recombination mutant (Fig. 6, lane R). These results suggest the existence of functional DSB repair pathways that likely operate during the exposure to the drug and later on to repair broken chromosomes, and confirm that the repair pathway(s) is (are) independent of Rad51 or Rad52. However, we cannot discard the possibility that survivors were derived from cells more resistant to bleomycin within the whole population.

The anti-tumor drug camptothecin inhibits topoisomerase I (Top1 in yeast) by stabilizing the covalent Top1-DNA intermediate that forms during the catalytic nicking-closing cycle of Top1 (Hsiang *et al*., 1985, 1989). This produces nicks in DNA. During the subsequent DNA replication, the nicks are processed to recombinogenic DSB (3´-phospho-tyrosyl-protein-blocked DSB), which are usually removed by Rad52-dependent recombination (Lettier *et al*., 2006). Both *rad51* and *rad52* nulls were more sensitive than the *rad59* null to several concentrations of camptothecin (0.5–2.5 μg/ml), whereas the latter behaved like parental CAF2 in both assays. Also, once again, the *rad52* null was more sensitive than the *rad51* null (Fig. 7, A and C). In agreement with a previous report (Legrand *et al*., 2007), the *rad52*-ΔΔ strain of *C. albicans* did not grow at 100 μM camptothecin, and the same was true for *rad51*, whereas *rad59* behaved again like parental CAF2 (not shown). None of the three genes, *RAD51*, *RAD52*, and *RAD59* was haploinsufficient with respect to camptothecin. Furthermore, reintegration of the cloned allele into either *rad51*-ΔΔ (Fig. 7A) or *rad52*-ΔΔ (not shown) restored the sensitivity of the parental strain CAF2. It should be mentioned that, following treatment with the several mutagenic agents, cells became growth-defective and yielded smaller colonies. Furthermore, as shown for *rad51*-ΔΔ cells treated with camptothecin (Fig. 7B), this defect increased with the dose or concentration of the mutagen. It is likely that cells that survive the corresponding treatment have undergone genetic rearrangements that decrease their growth rate, especially in the absence of Rad51 or Rad52. *C. albicans* and *S. cerevisiae* displayed a similar sensitivity to camptothecin, and the same was true for their respective *rad52* mutants, whereas *Scrad51* was significantly more sensitive than *Carad51* (Fig. 7C). This result supports once again the major role played by the Rad51-independent Rad52-dependent pathway of DNA repair in *C. albicans*.

d) Oxidative stress

Although the removal of lesions caused by ROS is primarily done through the base excision repair (BER) pathway (Memisoglu and Samson, 2000), HR has been shown to play also a role in survival of H_2O_2-damaged *S. cerevisiae* haploid cells in the absence of the piroxiredoxin Tsa1 (Huang and Kolodner. 2005). Quite surprisingly, a *rad52* mutant showed the same sensitivity as wild-type (Swanson *et al*., 1999), in spite of the fact that it exhibited an increase, although modest, in the rate of gross chromosomal rearrangements in response to H_2O_2 (Ragu *et al*., 2007). Also, direct interaction between human Ogg1 and Rad52 *in vitro* and *in vivo* has

been reported, indicating that *RAD52* cooperated with *OGG1* to repair oxidative damage (de Souza-Pinto et al., 2009).

Both *rad51* and *rad52* null mutants of *C. albicans* displayed slight sensitivity, to the oxidizing agents H_2O_2 (5 mM), menadione (0.1 M), and tBOOH (tetrabutyl hydrogen peroxide)(2mM), but *rad52* was slightly more affected, and its recovery from the oxidative damage after three days of incubation was lower. Reintegration of the respective genes in one the disrupted alleles restored the wild type sensitivity (not shown). The *rad59* null strain behaved as the parental CAF2 (Fig. 8). These results suggest that HR may be important for the recovery of the cells after extensive oxidative damage, again indicating that Rad52 plays a more relevant role than Rad51. We could not detect differences in sensitivities to 2mM tBOOH between *S. cerevisiae* and *C. albicans* in our plate-based assay, as reported by Legrand *et al.* (2008), even though we used the same diploid *S. cerevisiae* strains (BY4743 and derivatives). Both yeasts showed a similar sensitivity to all three oxidizing agents, but *rad52* of *C. albicans* was moderately more sensitive than its *S. cerevisiae* counterpart to tBOOH and 5 mM H_2O_2, indicating again a major role for this protein in the recovery of the corresponding lesions in *C. albicans*. It is likely that BER, or even NER, pathways (Torres-Ramos *et al*., 2000) account for most of the residual resistance to oxidizing agents observed in our experiments as well as for the recovery of *rad51* and *rad52* cells following long incubation periods.

DISCUSSION

In the present study, we have characterized *RAD51* and *RAD59* from *C. albicans*. The encoded proteins display extensive homology to proteins encoded by their respective orthologs from other eukaryotes. Also, as other eukaryotic Rad51p, CaRad51p showed a significantly higher homology to its counterpart from the *Archaea P. furiosus* than to RecA from *E. coli* as could be expected from the currently accepted tree of life.

Both heterozygous and null homozygous deletants of each gene were used to define their role in DNA repair. As described for *rad52*, *rad51* null strains were filamentous, and the same was true for the conditional *RAD51* strain under repressing conditions. We have reported that filamentation of *rad52* null strains is not dependent on Hypha-Specific-Genes (HSG) but appears to be linked to uncoupling of several events of the cell cycle which are likely derived from the accumulation of unrepaired DSBs (Andaluz *et al*., 2006). Furthermore, the accumulated lesions would need to be repaired by HR, since as described before (Andaluz *et al*., 2001; Legrand *et al*., 2008; our unpublished results), null strains of genes involved in NHEJ (Lig4, Ku80, Ku70) are not filamentous. The weaker filamentous phenotype of *rad51* strains as compared to *rad52* counterparts correlated with the fact that repair of DNA lesions caused by a variety of DNA-damaging agents is less dependent on Rad51 than on Rad52. In the same line, strains lacking Rad59, whose participation in DNA-repair was negligible, were not filamentous. Therefore, it is possible that the tendency towards the filamentation phenotype of HR specific recombination mutants in *C. albicans* reflects the relative importance of each protein in the repair of DSB or other type of DNA damage. An alternative explanation is that some HR proteins are themselves involved in preventing polar growth in *C. albicans*.

Karyotypes of *rad51* and *rad59* null strains were similar to those from CAI4 except for alterations in the size of ChrR. Furthermore, ChrR from one of the *rad59* null strains underwent changes in size throughout successive passages, and the same was true for both *rad51* strains. Since the ChrR bands from both CAI4 and a null *lig4* strain derived from were apparently stable through fifteen passages under the same conditions (Andaluz *et al*., 2002), it likely that Rad51- and Rad59-mediated HR events contribute to the stability of the rDNA repeats, and hence to maintaining the size of ChrR.

Both *RAD51* and *RAD52* showed clear haploinsuficiency in the repair of DNA damage caused by UV light. Haploinsufficiency was less pronounced when DNA damage was caused by bleomycin, and was negligible for MMS and camptothecin. One possibility is that the several types of lesions sequester different amounts of recombination proteins which apparently become unavailable for repair functions. On the other hand, *rad52*-ΔΔ strains of *C. albicans* are significantly more sensitive than *rad51*-ΔΔ counterparts to agents that cause DSBs (MMS, bleomycin and camptothecin) and oxidizing agents, an indication that Rad52 plays a prominent role during the HR processes used by *C. albicans* to repair the lesions caused by those agents. This suggests that the Rad51-independent pathway of DNA-repair may play a major role in this organism. Null homozygous *rad52* strains were also more sensitive to UV light than *rad51* counterparts but the difference in sensitivities was lower, suggesting that repair of the UV damage by HR preferentially uses the Rad51-dependent pathway of HR.

Interestingly, *rad59*-ΔΔ strains showed moderate sensitivity to UV light. UV doses that killed 80–85% of *rad51*-ΔΔ or *rad52*-ΔΔ cells respectively (25 J/m^2) also killed near 60% of *rad59*-ΔΔ cells, whereas only 5–10% of CAF2 cells lost viability. Therefore, Rad59 may play a role in the HR repair of pyrimidin dimers. By contrast, *rad59* cells were nearly as resistant as the parental CAF2 to MMS, bleomycin, camptothecin, or oxidizing agents. For instance, the *rad59*-ΔΔ strain showed wild type sensitivity at MMS concentrations (0.005%) that killed 55% and 85% of the *rad51* and *rad52* cells respectively. Similar results were obtained for bleomycin. At a dose of 10 μg/ml, survival was 0%, 10%, 78% and 90% for *rad52*-ΔΔ, *rad51*-ΔΔ, *rad59*-ΔΔ and CAF2 strains respectively. These values were 0%, 25%, 100% and 100% respectively at a dose of 2.5 μ/ml camptothecin. It should be noted, however, that higher concentrations of these agents (i.e., 0.01–0.02% MMS) that did not allow any growth of *rad51* or *rad52* null strains and moderately affected the parental strain CAF2, were deleterious to *rad59*-ΔΔ cells. This suggests that Rad59 participates significantly in the recovery after extensive damage. Furthermore, the participation of Rad59 in the recovery apparently depends on the nature of the lesions to be repaired. *C. albicans* was much more resistant to bleomycin than *S. cerevisiae*, and even *rad52* mutants of *C. albicans* were more resistant than *S. cerevisiae* wild type. This suggests that in addition to HR, *C. albicans* has evolved additional mechanisms to deal with the lesions caused by the anti-tumor agent bleomycin.

Interpretation of the effect of MMS on the recovery of cells after prolonged incubations was complicated by the instability of this compound. Still, our experiment comparing growth of *rad51* and *rad52* null strains from *C. albicans* and *S. cerevisiae* on fresh and aged MMS plates (Fig. 4C) allows us to making some speculations. For instance, we observed less growth of *Carad51* after four days of incubation on fresh MMS plates than after two days of incubation on the 2 day aged plates (i.e., the same age for MMS but half of the incubation time as compared with the first condition), whereas the opposite was true for *Scrad51*. Assuming that the number of lesions caused by MMS was similar for both yeast, one possibility is that following inoculation on fresh 0.005 % MMS plates more *Carad51* cells were irreversibly killed than *Scrad51* counterparts. Cells that were only inhibited, reassumed growth as MMS broke down and lesions could be more efficiently repaired. On the 2 day aged MMS plates, less *Carad51* cells died with drug, and survivors readily yielded a lawn that was even denser that its counterpart from *Scrad51*, due to the faster growth of *C. albicans*. A similar reasoning could be applied to *rad52* null mutants. Overall, our results strongly suggest that *S. cerevisiae* has evolved additional Rad51-independent and/or Rad52-independent mechanisms to repair the lesions caused by high concentrations of MMS (≥0.005%), whereas repair of the same lesions in *C. albicans* relies almost exclusively on Rad51 and Rad52. The obligate diploidy of *C. albicans* may have strengthened the homology dependency of most DSBs repair mechanisms, whereas other mechanisms that do not require homology, such as NHEJ, BER, or direct removal of methyl groups have become less efficient.

Construction and analysis of double mutants *rad51 rad59* and *rad59 rad52* will help to understand the specific mechanisms of HR that are involved in the repair of the lesions caused by several DNA-damaging agents in *C. albicans*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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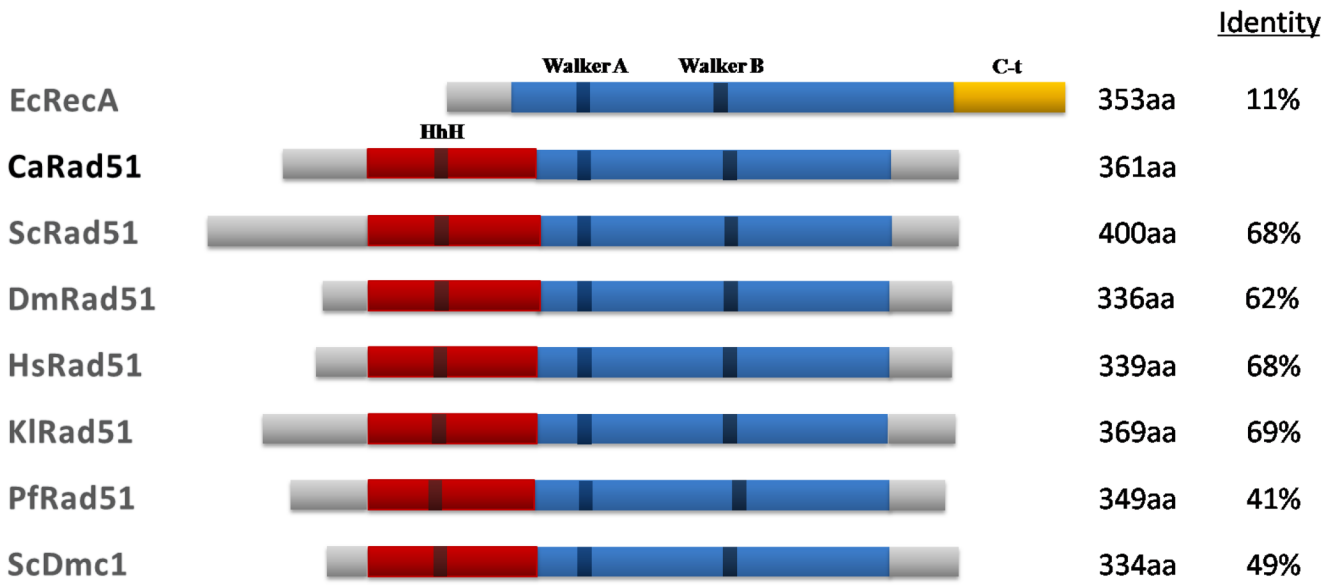

Fig. 1.

A comparison of the Rad51 proteins or homologues from the indicated species showing the relative locations of the conserved motifs. Percentages of identity are indicated at the right. For details, see text. *Ec, Escherichia coli, Ca, C. albicans, Sc, S. cerevisiae, Kl, Kluyveromyces lactis, Dm, Drosophila melanogaster, Hs, Homo sapiens, Pf, Pyrococcus furiosus*.

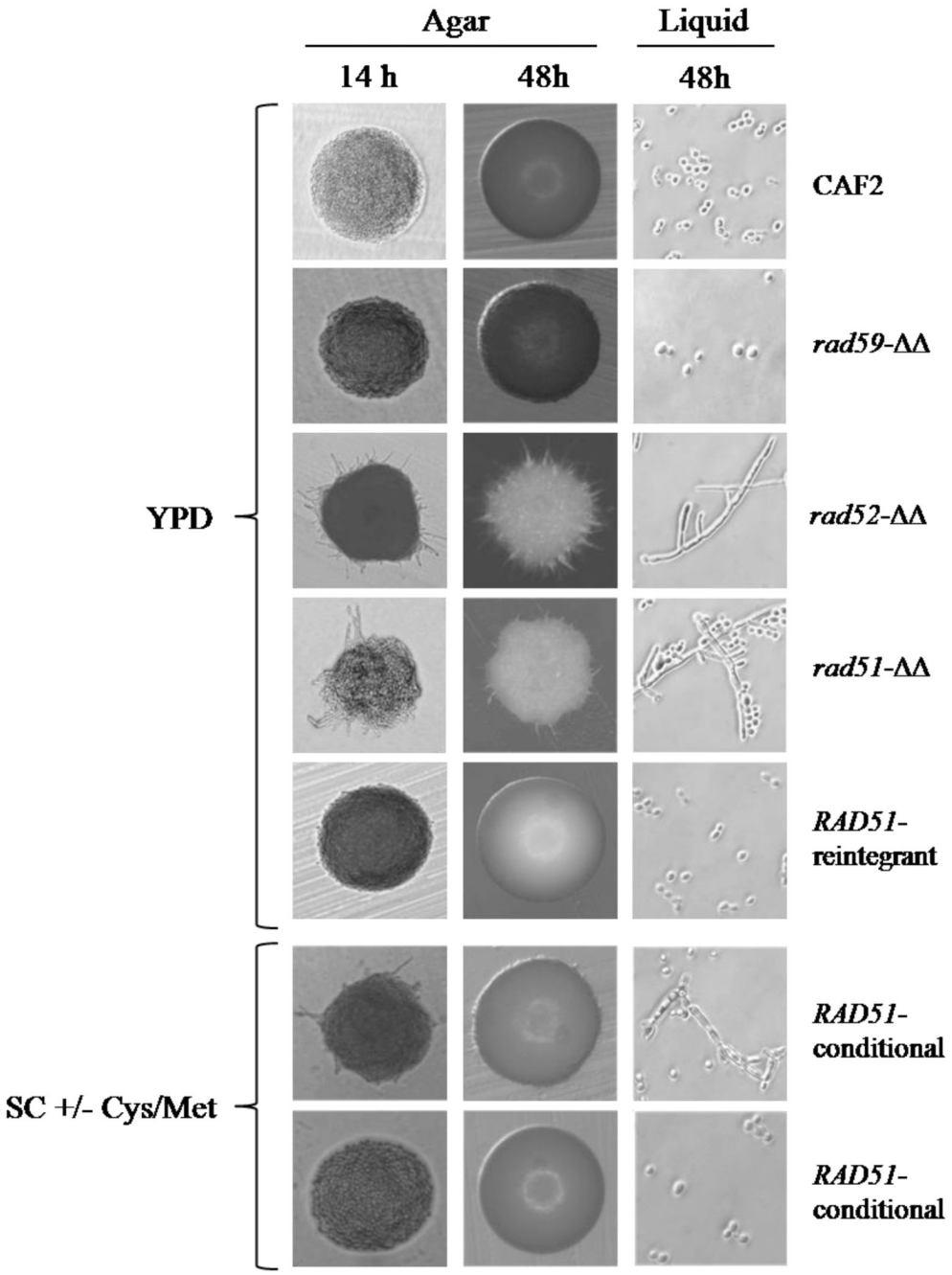

Fig. 2.

Colony and cell morphology of mutants. First and second columns: Colonies on YPD plates (CAF2, *rad51* -JGR5-, *rad59* -BNC1.1-, *rad52* -TCR2.1-, and *RAD51*-reintegrant) or SC plates with or without 2.5 mM Cys/Met, as indicated, were incubated for 14 h (second column) or 48 h (first column) and photographed under a Nikon Eclipse E600 optical microscope (×10) (second column) or a Nikon SMZ800 stereoscopic microscope (×2)(first column). Third column: cells were grown for 14h at 28°C in the same liquid media and photographed under an optical microscope (× 40). The other independent *rad51* null (JGR16) behaved like JGR5.

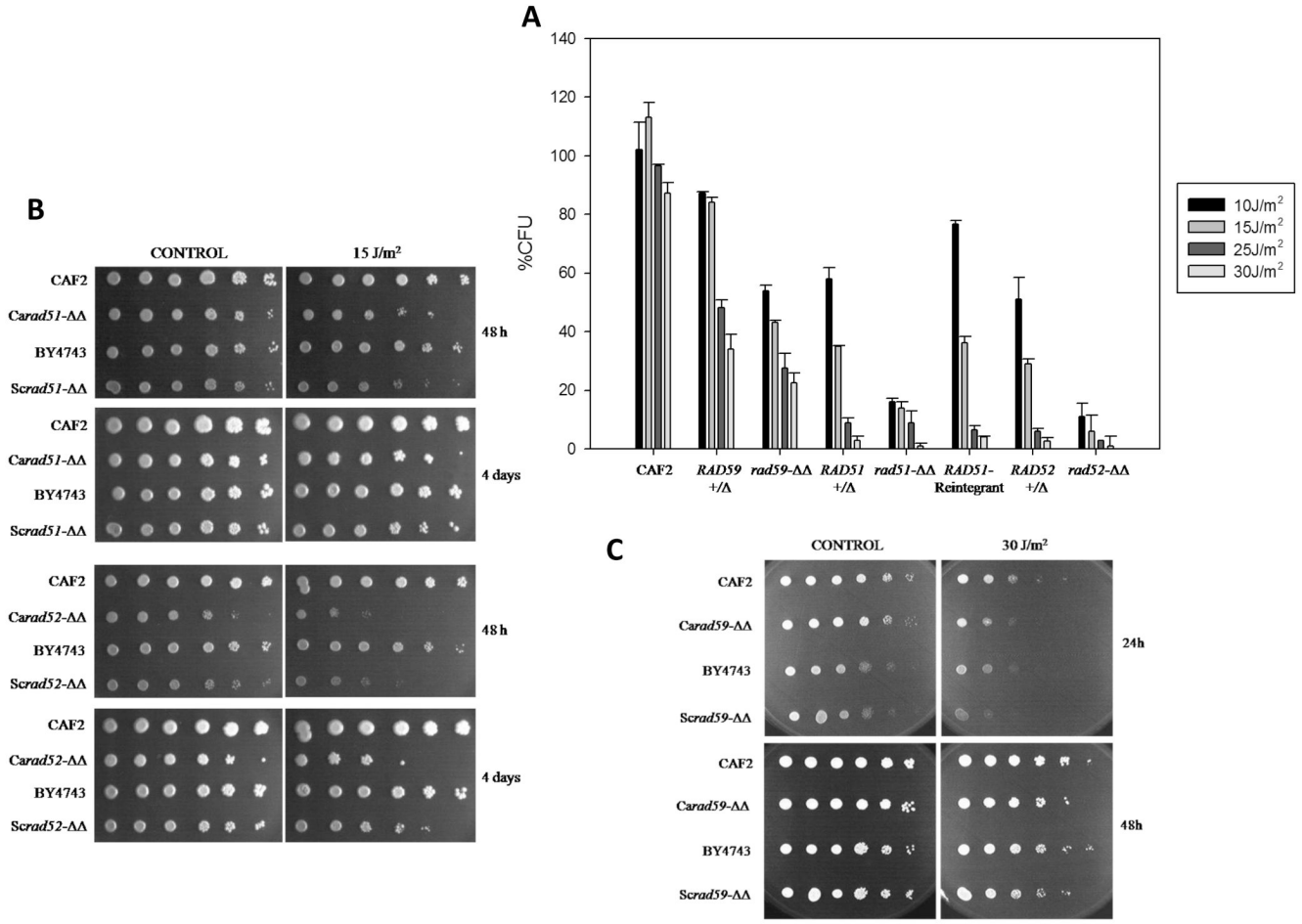

Fig. 3.

Sensitivity of the HR mutants of *C. albicans* to UV light. Following inoculation, YPD plates were irradiated with the indicated UV doses, as described in Materials ad Methods. **A**. Colony-forming units. About 350–400 cells were plated and results are expressed as percentages of survival relative to the corresponding untreated control. All plates were incubated at 30°C until colonies reached a diameter of about 1.5 mm. Colonies of *rad51* and *rad52* null strains were counted at one and two days after those of CAF2 (24 h). Also, because of their slower growth, colonies from irradiated cells were counted one or two days after the non-irradiated controls. Data are the mean of three independent experiments, each with duplicated samples. Error bars are shown. For each strain, control plates were grown similarly but not irradiated. Strains are as follows: CAF2 (*RAD51/RAD51*); JGR1 (*RAD51/rad51*Δ); TCR1 (*RAD52/rad52Δ*;); JGR5 (*rad51*-ΔΔ); BNC1 (*RAD59/rad59*Δ); BNC1.1 (*rad59*Δ*/rad59*Δ); TCR2.1 (*rad52*-ΔΔ), and JGR16A2 (*RAD51*-reintegrant). **B** and **C**. Comparison of sensitivities of *C. albicans* and *S. cerevisiae* and their respective HR mutants, *rad51, rad52* (**B**), and *rad59* (**C**), to UV using the serial dilution assay. Inoculated YPD plates were irradiated with UV (15 or 30 J/m^2, as indicated). Cells were incubated at 30°C for the indicated times

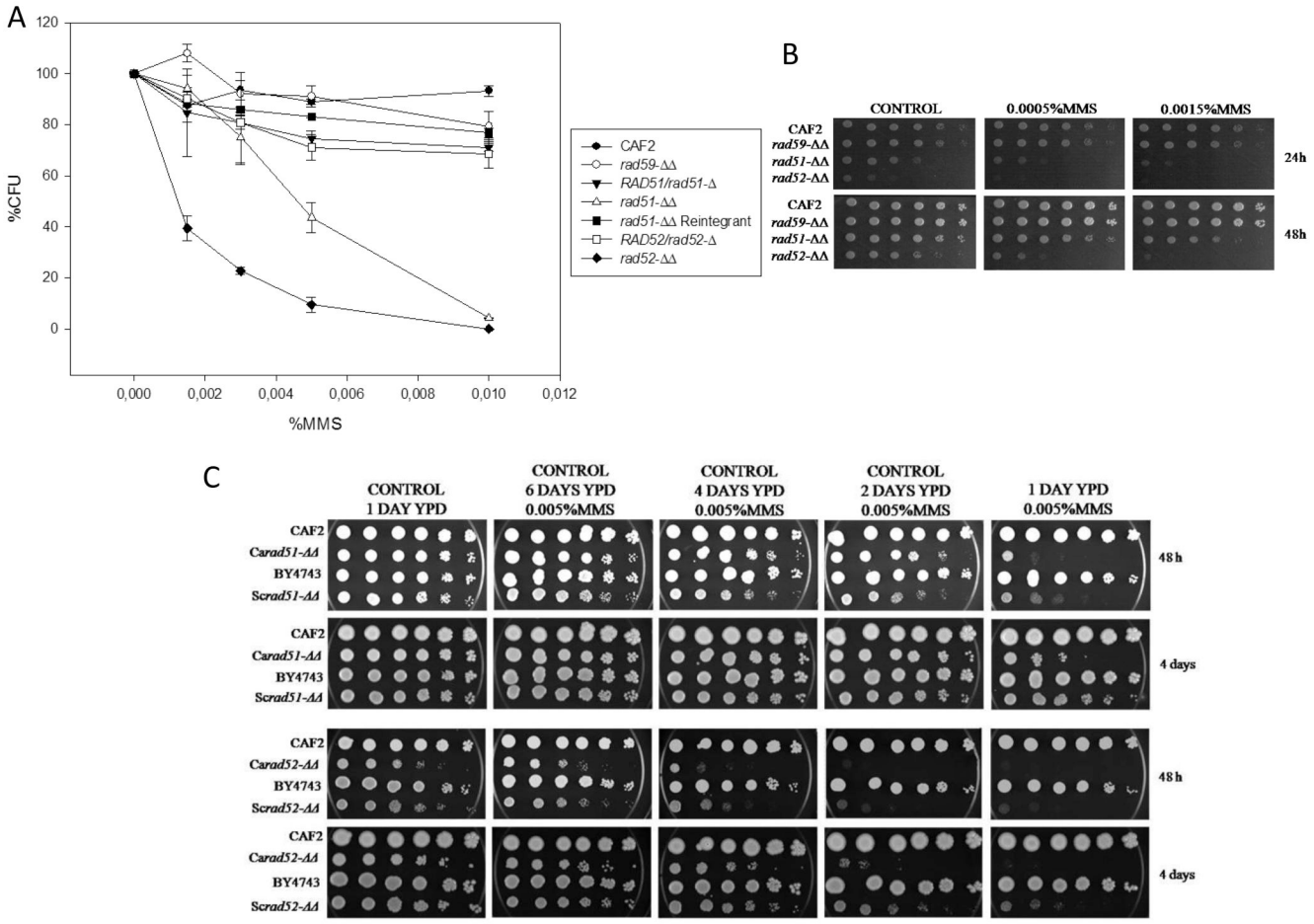

Fig. 4.

Sensitivity of the HR mutants of *C. albicans* to MMS. **A.** Colony-forming units. **B.** Serial dilution. **A.** About 450 cells were plated on YPD containing the indicated concentrations of MMS. All plates were incubated at 30°C until colonies reached a diameter of about 1.5 mm (see legend of Fig. 3). Because of their slower growth, colonies from treated cells were counted at one or two days after the untreated controls. Data are the mean of three independent experiments each one with duplicated samples. Error bars are shown. **B.** 5 μl of the initial cell suspension and serial fivefold dilutions were spotted onto YPD plates supplemented with the indicated concentrations of MMS. Plates were incubated at 30°C for the indicated periods and photographed. Strains were those of Fig. 3. **C.** Comparison of sensitivities of *C. albicans* and *S. cerevisiae* and their respective *rad51* and *rad52* null mutants to MMS using the serial dilution assay. YPD plates had been supplemented with 0.005% MMS and allowed to age for the indicated periods before inoculation of cells; plates on the right column contained fresh MMS. All the plates were incubated at 30°C in the dark for the indicated times.

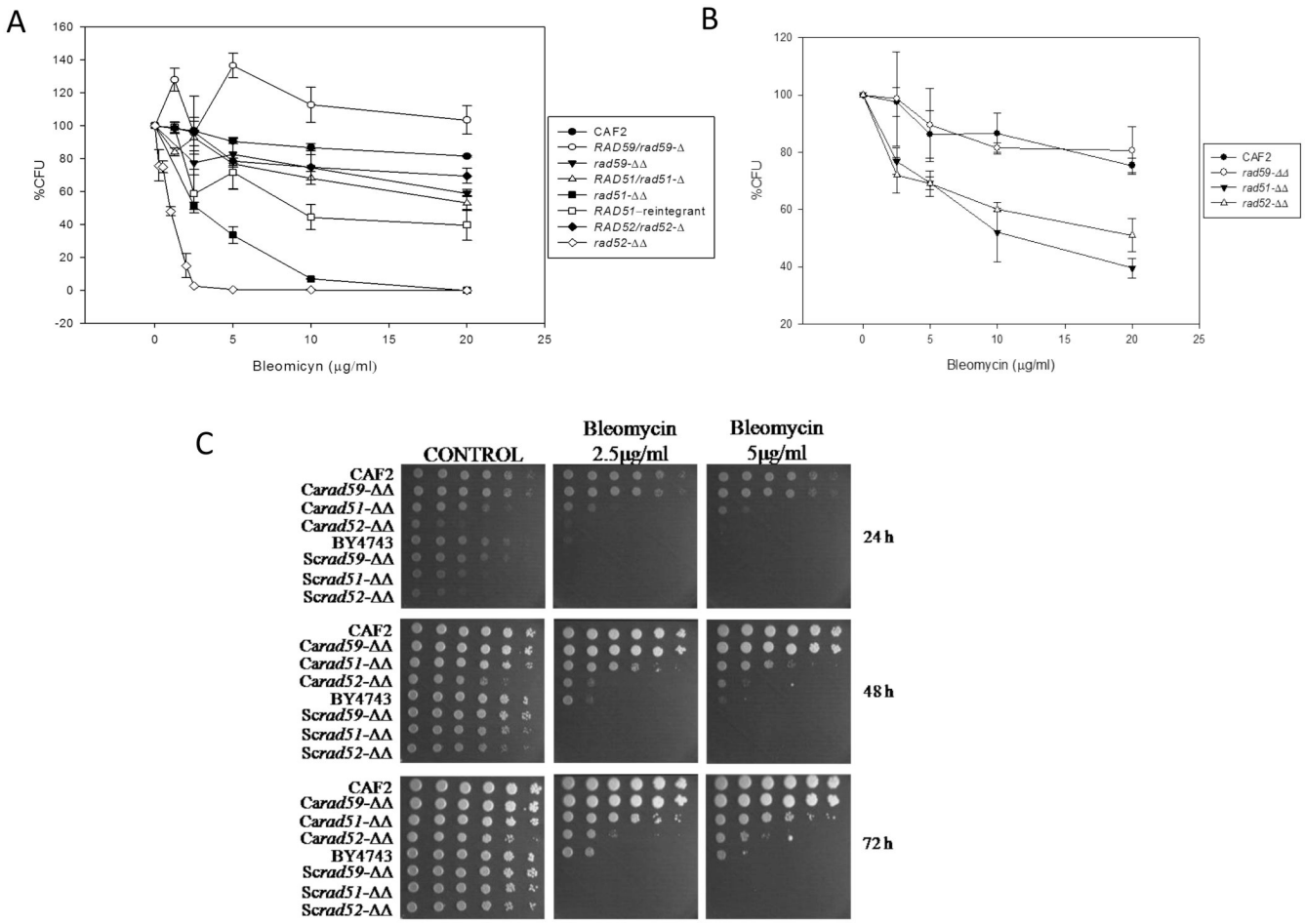

Fig. 5.

Sensitivity of the HR mutants of *C. albicans* to bleomycin. **A.** Colony-forming units (Materials and Methds). For details, see the Fig. 4 legend. Three independent experiments were performed. Error bars are shown. **B.** Cells were incubated for 2 h in liquid YPD medium supplemented with the indicated concentrations of bleomycin, washed and the number of survivors (CFU) determined on YPD plates lacking bleomycin. Plates were incubated at 30°C until colonies are visible (see legend of Fig. 4) and counted. Data are the mean of three independent experiments, each one with duplicated samples. Error bars are shown. **C.** Comparison of sensitivities of *C. albicans* and *S. cerevisiae* and their respective HR mutants to bleomycin using the serial dilution assay. YPD plates had been supplemented with 2.5 or 5 µg/ml of bleomycin. Plates were incubated at 30°C for the indicated times.

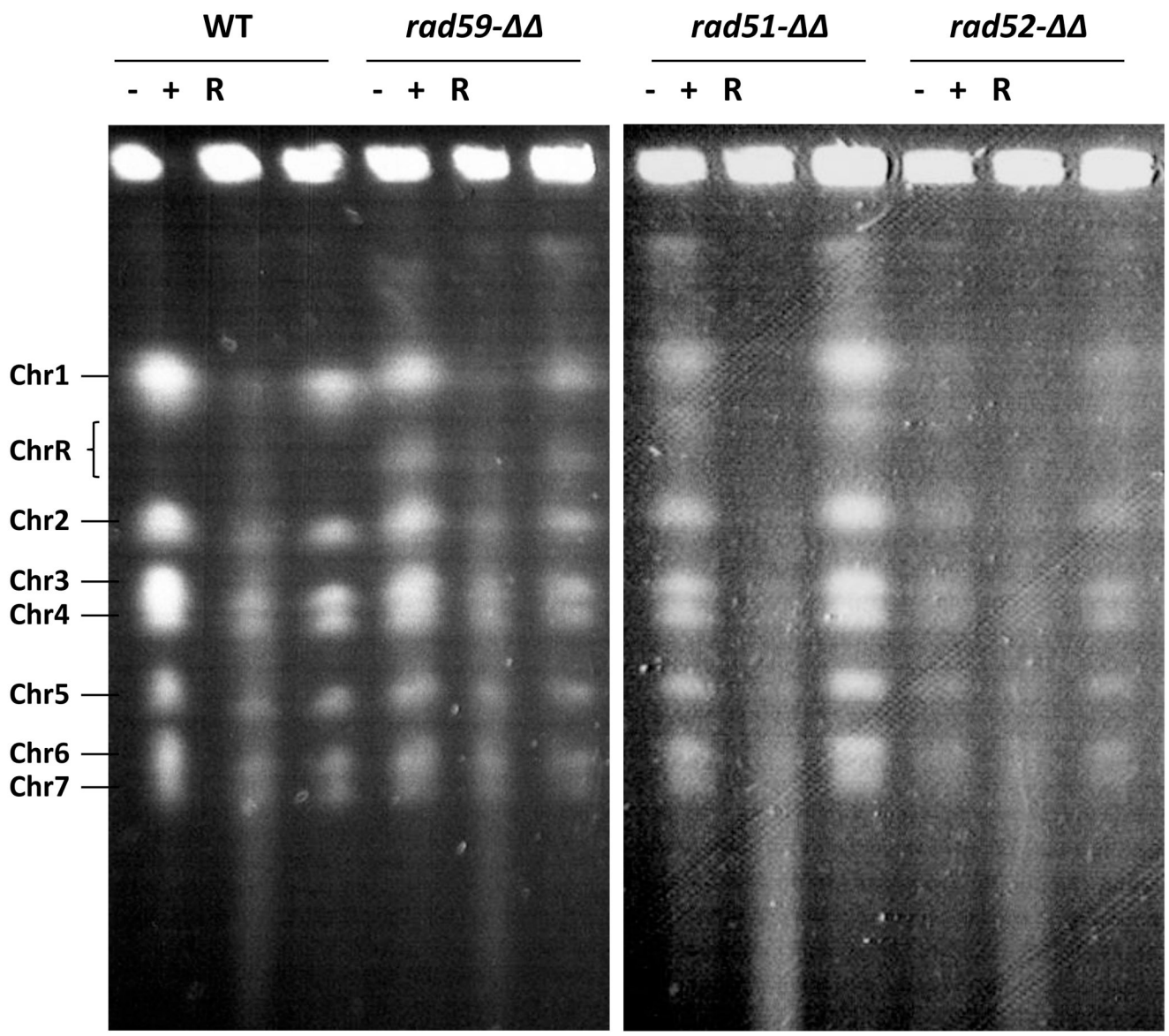

Fig. 6.

Chromosomal alterations induced by bleomycin in *C. albicans* CAF2 and the indicated HR mutants. Cells were treated (+) or not (−) with 20 μg/ml bleomycin. After one hour, they were washed and karyotypes determined. An aliquot of each bleomycin treated sample was inoculated on YPD plates. After 48 h, one of the developing colonies was picked, grown in liquid YPD and processed for determination of karyotypes as before (R).

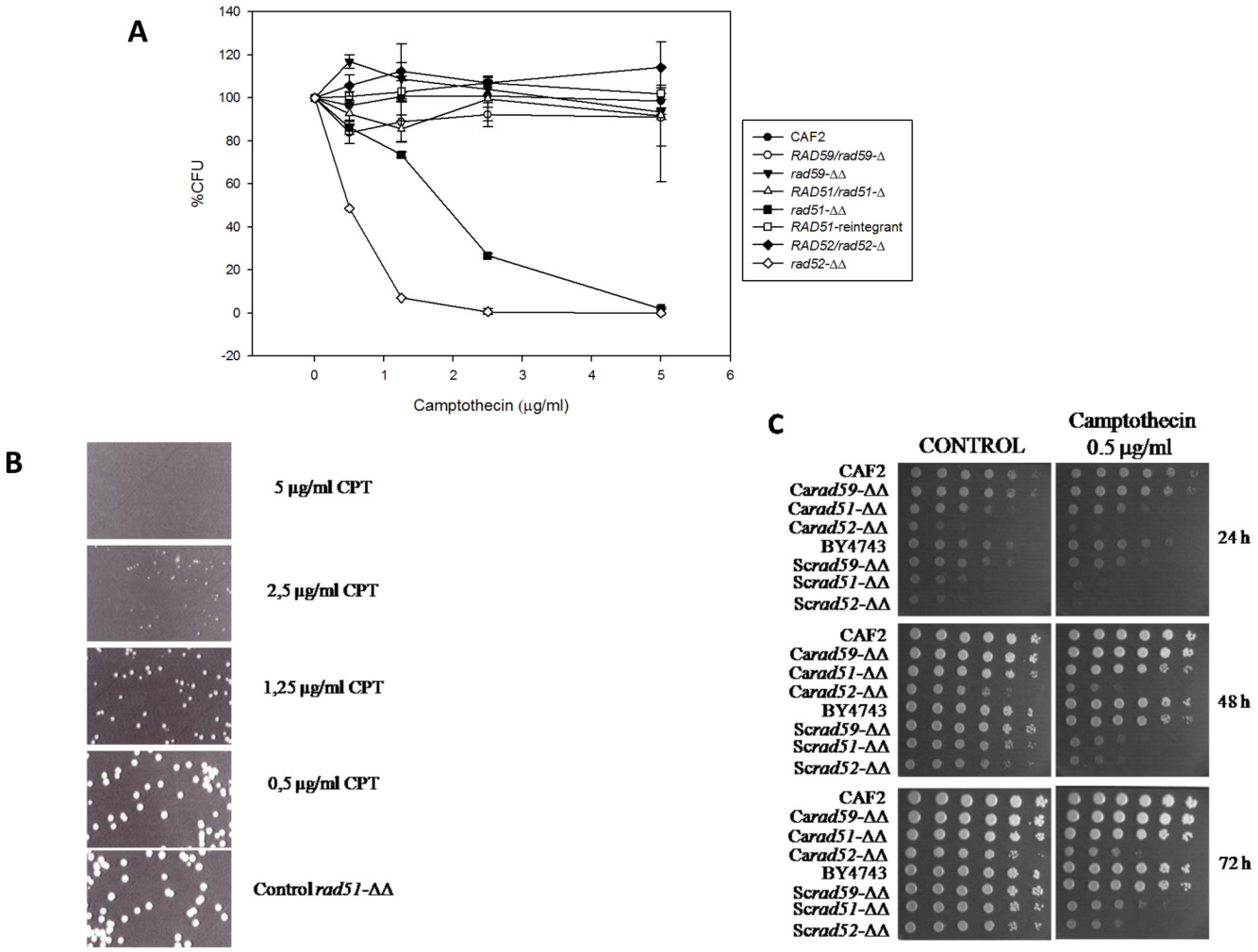

Fig. 7.

Sensitivity of the HR mutants of *C. albicans* to camptothecin. **A.** Colony-forming units. For details, see the Fig. 4 legend. Data are the mean of two independent experiments each one with duplicated samples. Error bars are shown. **B.** Colony size-dependency on camptothecin concentration. Null *rad51*, JGR5, cells were inoculated on YPD plates supplemented with the indicated concentrations of camptothecin, incubated for 72h and photographed. **C.** Comparison of sensitivities of *C. albicans* and *S. cerevisiae* and their respective HR mutants to 0.5 µg/ml of camptothecin using the serial dilution assay. For details, see Fig. 4.

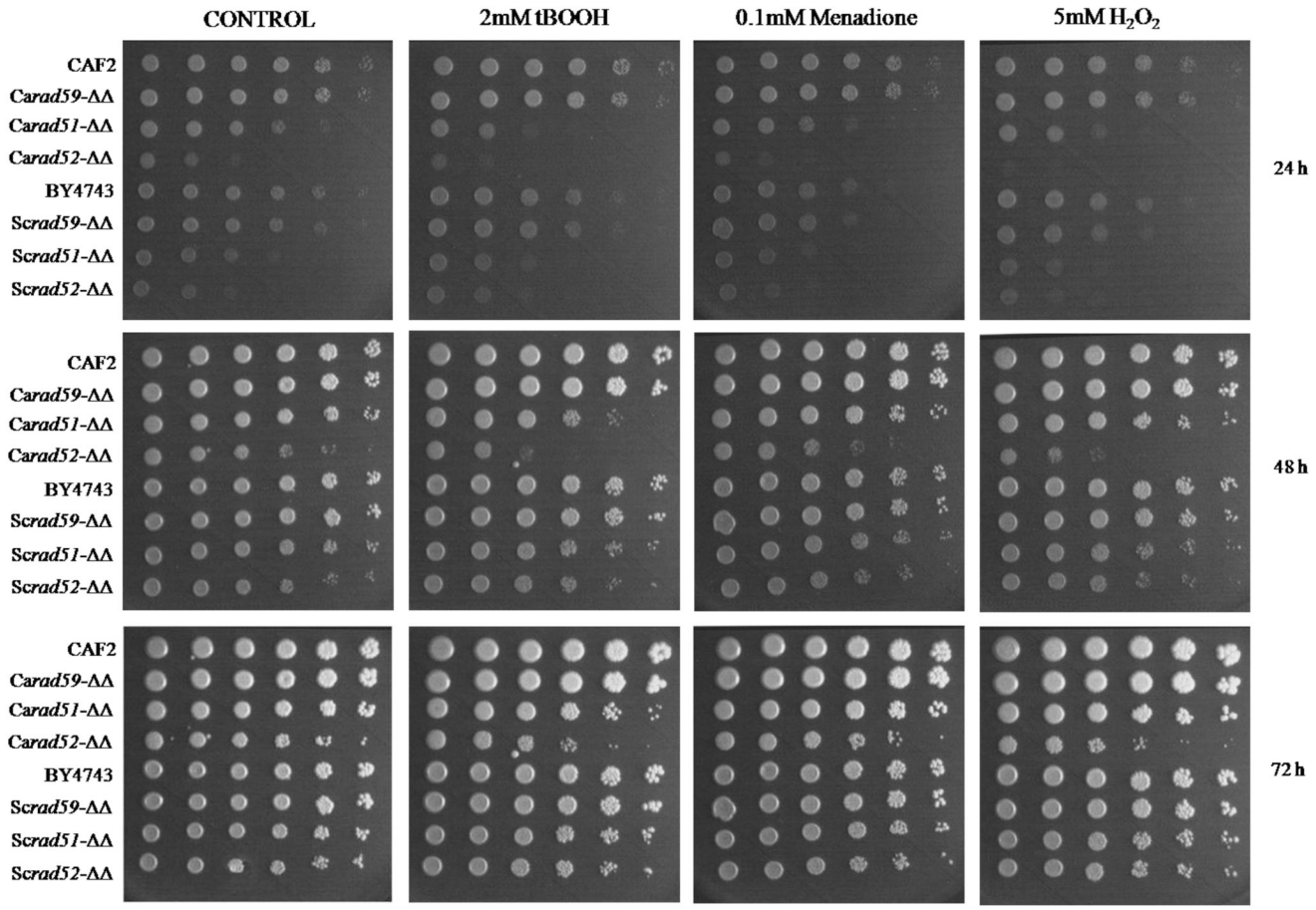

Fig. 8.

Comparison of sensitivities of *C. albicans* and *S. cerevisiae* and their respective HR mutants to oxidizing agents using the serial dilution assay. For details, see Fig. 4.

Table 1

Strains used in this study.

Strain	Parental	Genotype	Reference
SC5314		Wild type	Gillum *et al*. (1984)
CAF2	SC5314	*ura3::imm434/URA3*	Fonzi and Irwin (1993)
CAI4	CAF2	*ura3::imm434/ura3::imm434*	Fonzi and Irwin (1993)
JGR1	CAI4	*ura3::imm434/ura3::imm434 RAD51/rad51::hisG-URA3-hisG*	This work
JGR1.1	JGR1	*ura3::imm434/ura3::imm434 RAD51/rad51::hisG*	This work
JGR2	CAI4	Isogenic to JGR1	This work
JGR2.1	JGR2	Isogeneic to JGR1.1	This work
JGR5	JGR1.1	*ura3::imm434/ura3::imm434 rad51::hisG/rad51::hisG-URA3-hisG*	This work
JGR5A	JGR5	*ura3::imm434/ura3::imm434 rad51::hisG/rad51::hisG*	This work
JGR16	JGR2.1	Isogenic to JGR5	This work
JGR16A	JGR16	Isogenic to JGR5A	This work
JGR25	JGR16A	*ura3::imm434/ura3::imm434 rad51::hisG/RAD51-URA3-hisG*	This work
CGR1.1	JGR1.1	*Δura3::imm434/Δura3::imm434 Δrad51::hisG/Δrad51:: MET3-RAD51-URA3*	This work
CGR2.1	JGR2.1	Isogenic to CGR1.1	This work
TCR1	CAI4	*ura3::imm434/ura3::imm434 RAD52/rad52::hisG-URA3-hisG*	Ciudad *et al*. (2004)
TCR2.1	TCR1.1	*ura3::imm434/ura3::imm434 rad52::hisG/rad52::hisG-URA3-hisG*	Ciudad *et al*. (2004)
TCR2.1.1	TCR2.1	*ura3::imm434/ura3::imm434 rad52::hisG/rad52*	Ciudad *et al*. (2004)
BNC1	CAI4	*ura3::imm434/ura3::imm434 RAD59/rad59 ::hisG-URA3-hisG*	This work
BNC2	CAI4	Isogenic to BNC1	This work
BNC1.1	CAI4	*ura3::imm434/ura3::imm434 rad59::hisG/rad59 ::hisG-URA3-hisG*	This work
BNC2.1	CAI4	Isogenic to BNC1.1	This work
S. cerevisiae BY4743		*MAT a/alpha; his3Δ1/ his3Δ1; leu2Δ0/ leu2Δ0; lys2Δ0/LYS2; MET15/met15Δ0; ura3Δ0/ ura3Δ0*	Euroscarf
S. cerevisiae *rad51*	BY4743	*MAT a/alpha; his3Δ1/ his3Δ1; leu2Δ0/ leu2Δ0; lys2Δ0/LYS2; MET15/met15Δ0; ura3Δ0/ ura3Δ0; Δrad51::kanMX4/ Δrad51::kanMX4*	Euroscarf
S. cerevisiae *rad52*	BY4743	*MAT a/alpha; his3Δ1/ his3Δ1; leu2Δ0/ leu2Δ0; lys2Δ0/LYS2; MET15/met15Δ0; ura3Δ0/ ura3Δ0; Δrad52::kanMX4/ Δrad52::kanMX4*	Euroscarf
S. cerevisiae *rad59*	BY4743	*MAT a/alpha; his3Δ1/ his3Δ1; leu2Δ0/ leu2Δ0; lys2Δ0/LYS2; MET15/met15Δ0; ura3Δ0/ ura3Δ0; Δrad59::kanMX4/ Δrad59::kanMX4*	Euroscarf