Partner Choice in Spontaneous Mitotic Recombination in Wild Type and Homologous Recombination Mutants of Candida albicans

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During normal cell proliferation, spontaneous DNA lesions arise at measurable rates and their frequency is significantly increased by the presence of environmental compounds generally referred to as genotoxins. For instance, humans are estimated to generate up to $10^5$ mutations/cell/day (Hoeijmakers 2009). To repair DNA lesions, cells have evolved a variety of mechanisms that remove damage and accurately restore genetic information (Boiteux and Jinks-Robertson 2013; Wyrick and Roberts 2015). However, repair may also cause genomic rearrangements whose location and frequency are influenced by the genome structure, particularly by the presence of repetitive elements (Chan and Kolodner 2011). Repeated copies of DNA segments are potential targets for homologous recombination (HR) if resection of double strand breaks (DSB) exposes the complementary sequences (Aguilera et al. 2000; Prado et al. 2003; Heyer et al. 2010; Symington et al. 2014).

Single-strand annealing (SSA) plays a major role in direct-repeat recombination resulting in the loss of one repeat and the intervening sequence (Klein et al. 2019). Studies in haploid Saccharomyces cerevisiae (S. cerevisiae) strains on DSB-induced repeat recombination have shown that SSA was dependent on the annealing activity of Rad52 for repeat length of 1-2 kb (Rudin and Haber 1988; Fishman-Lobell et al. 1992; Sugawara and Haber 1992; Jablonovich et al. 1999) but not when repeats were much larger (e.g., CUP1 gene or rRNA gene arrays) (Ozenberger and Roeder 1991). Additional work revealed that this process was significantly impaired in the absence of RAD59, a RAD52
paralog, especially when the direct repeats were short (40-fold for 205 bp repeats) (Petukhova et al. 1999; Sugawara et al. 2000; Davis and Symington 2001, 2003; Wu et al. 2006; Pannunzio et al. 2008). In the presence of Rad52 and Rad59, DSB-induced SSA utilized repeats as short as 29 bp and showed linear dependency on the length of homologous repeats up to 415 bp (Ivanov et al. 1996).

Non-DSB direct-repeat recombination (spontaneous) via SSA-like mechanisms can also lead to loss of one repeat plus the intervening sequence. In S. cerevisiae, the rate of spontaneous direct-repeat recombination (not DSB-induced) was directly proportional to the substrate length and the minimal repeat length for efficient recombination was 285 bp; some recombination was detected for 80 bp repeats but not for 37 bp repeats (Jinks-Robertson et al. 1993). This suggests the existence of specific differences between DSB-induced and spontaneous direct-repeat recombination via SSA. Importantly, SSA does not require strand invasion and is therefore independent of Rad51 (and its paralogs Rad55 and Rad57) (Ivanov et al. 1996; Jablonovich et al. 1999; Pannunzio et al. 2008).

The genome of C. albicans, the most common fungal pathogen, is particularly rich in direct repeats (Braun et al. 2005; Smich et al. 2012; Todd et al. 2019). Not much is known in C. albicans about the recombination pathways involved in repeat number alteration and the potential consequences for overall genome structure and host-fungus interactions. It is believed that repeat number alterations are caused by replication slippage and recombination and may provide an evolutionary advantage in fluctuating environments thereby providing the population with a selection of proteins with different properties. Not only may these mechanisms alter repeat numbers and generating novel alleles of a specific ORF, recombination between repeats of two genes from the same family (i.e., agglutinin-like (ALS) sequence gene family in C. albicans) could lead to chimera formation, which may be endowed with novel properties advantageous for survival in the host (Zhang et al. 2003; Zhao et al. 2011). Several studies have shown that repeats within coding regions of genes may have functional roles. For example, the repeat copy number in ALS5 directly affects adhesion to fibronectin (Raucos et al. 2006). Repeats of Hwp1, Pir1 and Eap1 are important in adhesion to buccal epithelial cells (Staab et al. 2004), protein localization (Sumita et al. 2005), and positioning of binding sites to several materials and cells, respectively (Li and Palecek 2008). Furthermore, repeat length variation in cell wall-associated proteins may contribute to the overall antigenic variation in C. albicans, which in turn aids in adaptation to and evasion from the host (Verstrepen and Fink 2009; Zhang et al. 2010; Zhao et al. 2011; Zhou et al. 2017).

Here, we took advantage of the URA-Blaster cassette which consists of the URA3 gene of C. albicans flanked by 1.1 kb hisG direct repeats (Alani et al. 1987; Fonzi and Irwin 1993). To study direct-repeat recombination and to test for allele-specific effects, we replaced each allele of RAD52 (located on the left arm of chromosome 6 (Chr6) with this cassette, measure rates of URA3 loss as resistance to 5-fluoroorotic acid (5FOA)), and then analyzed 5FOA derivatives by CHEF Southern and/or Southern blot analyses as previously described (Ciudad et al. 2004; Bellido et al. 2015). To isolate 5FOA derivatives, a single colony from the indicated genetic background was re-isolated on an YPD plate and then streaked on a new YPD plate supplemented with 0.1% (w/v) 5FOA and 25 μg/ml uridine, since C. albicans ura3 mutants are fed with uridine. To disrupt RAD52 with the SAT1-flipper cassette, the upstream and downstream regions of the RAD52 ORF were PCR-amplified from genomic DNA of strain CAF2-1, using oligonucleotides RAD52F-Apal/RAD52R-Xhol and RAD52F-SacI/RAD52R-SacI respectively (Fig. S1 and Table S2). Amplified fragments were cloned in pSF216 plasmid flanking the SAT1-flipper cassette. The disruption cassette was released by digestion with Apal and SacI and transformed into the indicated hemizygous strains RAD52/Δrad52Δ:hisG-URA3-hisG (Figure 1) using a MicroPulser Electroporator system (Bio-Rad) (Ciudad et al. 2016). Nourseothricin-resistant (NouR) colonies were selected on YPD plates supplemented with 200 μg/ml nourseothricin. Several transformants were initially selected based on their thorny colonies and filamentous cell morphology, two phenotypes of null rad52 strains (Andaluz et al. 2006) and then PCR verified for both integration of the SAT1-Flipper cassette in the RAD52 locus (oligonucleotides SAT1F-Flip/RAD52R) and absence of any residual RAD52 allele (oligonucleotides RAD52F-IF/RAD52R-IR). SAT1 loss was induced by overnight growth in liquid YPM (2% maltose, 1% yeast extract, 2% bactopeptone) (Reuss et al. 2004). The resulting nourseothricin-sensitive (NouS) derivatives were selected as small colonies on YPD plates supplemented with 20 μg/ml nourseothricin. They were verified by PCR for SAT1 loss (oligonucleotides RAD52F-R and RAD52-R). These strains carry the rad52::FRT allele (FRT strains) (Fig. S1).

To determine URA3 loss rates in the presence of both RAD52 alleles we used the SHE9/shc::hisG-URA3-hisG reporter. SHE9, located in Chr2L (coordinates 615,050 – 616,624), is a non-essential gene whose null homozygous disruptant (Fig. S2) does not show any obvious phenotype (Andaluz et al. 2001b) (our unpublished results).

**DNA extraction and analysis**

Extraction of genomic DNA, preparation of chromosomes, and CHEF Southern hybridization have been described (Andaluz et al. 2011). Two different PFGE protocols were used. In the first protocol (short run), all chromosomes were separated. The second protocol separates both homologs of Chr7 and, in some strains, of Chr6 (Andaluz et al. 2011). To test for the presence of one or both homologs of Chr6 we used the SNP status (genotype) of multiple markers along chromosomes as proxy. Routine SNP-RFLP analyses were carried out as described (Forche et al. 2009) using the indicated primers (Table S2).

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**MATERIALS AND METHODS**

**C. albicans strains used in this study**

Single and double mutant strains used in this work were generated from strain CAI4, a Ura- derivative of the reference strain SC5314 (Gillum et al. 1984), by disrupting the indicated allele with the hisG-URA3-hisG cassette flanked by promoter and terminator regions of the target gene (Table S1). Transformants were verified by PCR and/or Southern blot analyses as previously described (Ciudad et al. 2004; Bellido et al. 2015). To isolate 5FOA derivatives, a single colony from the indicated genetic background was re-isolated on an YPD plate and then streaked on a new YPD plate supplemented with 0.1% (w/v) 5FOA and 25 μg/ml uridine, since C. albicans ura3 mutants are fed with uridine. To disrupt RAD52 with the SAT1-flipper cassette, the upstream and downstream regions of the RAD52 ORF were PCR-amplified from genomic DNA of strain CAF2-1, using oligonucleotides RAD52F-Apal/RAD52R-Xhol and RAD52F-SacI/RAD52R-SacI respectively (Fig. S1 and Table S2). Amplified fragments were cloned in pSF216 plasmid flanking the SAT1-flipper cassette. The disruption cassette was released by digestion with Apal and SacI and transformed into the indicated hemizygous strains RAD52/Δrad52Δ:hisG-URA3-hisG (Figure 1) using a MicroPulser Electroporator system (Bio-Rad) (Ciudad et al. 2016). Nourseothricin-resistant (NouR) colonies were selected on YPD plates supplemented with 200 μg/ml nourseothricin. Several transformants were initially selected based on their thorny colonies and filamentous cell morphology, two phenotypes of null rad52 strains (Andaluz et al. 2006) and then PCR verified for both integration of the SAT1-Flipper cassette in the RAD52 locus (oligonucleotides SAT1F-Flip/RAD52R) and absence of any residual RAD52 allele (oligonucleotides RAD52F-IF/RAD52R-IR). SAT1 loss was induced by overnight growth in liquid YPM (2% maltose, 1% yeast extract, 2% bactopeptone) (Reuss et al. 2004). The resulting nourseothricin-sensitive (NouS) derivatives were selected as small colonies on YPD plates supplemented with 20 μg/ml nourseothricin. They were verified by PCR for SAT1 loss (oligonucleotides RAD52F-R and RAD52-R). These strains carry the rad52::FRT allele (FRT strains) (Fig. S1).

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Generation, verification and characterization of Chr6A and Chr6B tester strains

In the strain background used in this study, Chr6 homologs exhibit size differences sufficient for separation on CHEF gels. Chr6 homolog length polymorphisms can be due to differences in the number of repeats either within the major repeat sequence (MRS) (Chibana and Magee 2009) or within members of the ALS family (ALS6, ALS1, ALS10, ALS5, and ALS2) located on this chromosome (Zhang et al. 2003; Zhao et al. 2011).

We used strains heterozygous for RAD52 and rad52 null strains to generate tester strains with the hisG-URA3-hisG construct either replacing RAD52 on the A or the B homolog (Table S1). CAGL4A and CAGL4.1A are two independent rad52 Ura+ derivatives of the heterozygous parental, CAGL1B (Ciudad et al. 2004). We have previously shown that CAGL4A and CAGL4.1A conserved both homologs of Chr6 (Andaluz et al. 2011). To identify the test homolog in CAGL4A and CAGL4A.1, we first performed a physical analysis of Chr6 homologs present in its parental heterozygous strain CAGL1B.1 (Ura-).

CHEF Southern hybridization with a COX12 probe confirmed the presence of both Chr6 homologs in CAF2-1, CAI4, and CAGL1B.1, and hybridization with RAD52 and hisG probes localized RAD52 to the smaller Chr6 homolog (Chr6A) and rad52:hisG to the larger homolog (Chr6B) (Fig. S3A). In agreement with this, a spontaneous His- derivative (GLH1-7) of a rad52 strain (TCR2.1.1) disomic for Chr6 only carried the small homolog and was homozygous, haplotype A, for multiple Chr6 SNPs markers (Forche et al. 2009; Andaluz et al. 2011).

We also took advantage of heterozygosity within the RAD52 ORF to identify the RAD52 allele present in each heterozygote using SNP/RFLP. A 793 bp region of the RAD52 ORF was amplified with primers RAD52_501F and RAD52_1290R (Table S2) and subjected to a restriction digest with TaqI. This enzyme cuts twice in allele A (RAD52A) yielding 3 restriction fragments (251 bp, 237 bp, 305 bp) and once in allele B (RAD52B) resulting in 2 restriction fragments (251 bp and 542 bp) (Fig. S3B). As expected, both alleles were detected in strain CAI4 (as well as in parental strains SC5314 and CAF2-1, not shown) whereas CAGL1B was homozygous for RAD52A (Fig. S3B). We concluded that during the generation of CAGL4A and CAGL4A.1, the RAD52B allele present in the larger Chr6 (Chr6B) of CAI4 strain was disrupted first resulting in the intermediate strain CAGL1B (test chromosome B) (Fig. S3, top). Because of previous findings that the Chr6B allele may harbor recessive lethal alleles (and therefore cannot be lost) (Andaluz et al. 2011; Hickman et al. 2013; Feri et al. 2016), new strains were generated with the URA-Blaster inserted carrying Chr6A as the test chromosome (CAGL1A). These strains were used to generate rad52:hisG strains with the opposite configuration, i.e., if derived from CAGL1A, test chromosome was Chr6B, or rad52::FRT strains that conserved the parental configuration, i.e., if derived from CAGL1A, Chr6A remained as test chromosome (Figure 1, Table S1). All heterozygous and null RAD52 strains were tested for the presence of both

![Figure 1](https://example.com/figure1.png)

**Figure 1** Diagrams. (A) Approach used in this work to generate tester strains with the hisG-URA3-hisG cassette either on allele A (cyan) or allele B (magenta) at the RAD52 locus on Chr6L. (B) Chromosome 6 homologs A (cyan) and B (magenta) and location of SNPs markers 122, 123, (left arm), including their distances from the left telomere, CEN6 (cen), and SNP marker 132 (right arm). In panel (A) the alternative allele in rad52 null strains was either a hisG fragment or the FRT site resulting from the eventual excision of the SAT1 cassette, as indicated.
Chr6 homologs by SNP RFLP (SNP122, SNP123 and SNP132) and for
the lack of obvious GCR by PFGE (Fig. S4). We concluded that all of
them were appropriate for the generation and subsequent genetic analysis
of the 5FOA\(^R\) derivatives.

**Fluctuation test**

Strains were streaked to single colonies on YPD and incubated for 2 - 4
days at 30\(^\circ\). At least 10 independent colonies from each strain were
resuspended in 100 \(\mu\)l of sterile water. Tenfold dilutions were generated
using 10 \(\mu\)l of the initial resuspension and 40 \(\mu\)l of the 10 \(^{-4}\) dilution
were spotted onto YPD plates to determine the total amount of CFUs.
The remaining 90 \(\mu\)l of the initial resuspension was spread onto 5FOA
plates. Alternatively, fluctuation analysis using twenty overnight (16 h)
liquid cultures seeded with single colonies was done as described by
Forche et al. (2011). Importantly, for wild type strain CAGL1B, URA3
loss rates (5FOA\(^R\)) were similar for both methods (1.5 \(\times\) 10\(^{-5}\)/cell
generation for colonies vs. 2.5 \(\times\) 10\(^{-5}\)/cell generation for liquid
cultures). Therefore, fluctuation analyses were carried out using the former
protocol. YPD and 5FOA plates were incubated at 30\(^\circ\) for 3 days and
colonies were counted. URA3 loss rates were calculated as described
(Forche et al. 2011).

**Molecular characterization of URA3 loss in 5FOA\(^R\) derivatives**

For most strains, a minimum of 20 5FOA\(^R\) derivatives per strain back-
ground were analyzed. A scheme with the several steps for character-
ization of the 5FOA\(^R\) derivatives at the RAD52 locus is shown in Fig. S4.
SNP results are summarized in Table S3.

We used \textit{S. cerevisiae} chromosomal markers to determine the size of
SNCs (Argueso et al. 2008). The calculated size correlated well with the
genotypes of markers snp122 and snp132, which are 832 kb and 545 kb
away from the right telomere, respectively. SNCs from strains hetero-
ygous for snp122 should be larger than 832 kb, whereas SNCs from
strains homozygous for both snp122 and snp123 should be smaller
than 545 kb. Importantly, all strains carrying SNCs were heterozygous
for snp123 marker, an indication that no SCN was smaller than 545 kb
(Figure 1). Fisher’s exact test was used to determine whether the fre-
quency of different loss mechanisms in the mutant strains vs. wild type
were significant (p value of < 0.05).

The occurrence of SSA at the SHE9 locus was investigated by PCR
using primers SHE1 and SHE2, which amplify bands of 845 bp and
1171 bp for SHE9 and hisG repeat respectively, whereas the presence
of URA3 in 5FOA\(^R\) segregants (URA3 mutational inactivation) locus
was verified using primers SHE1 and URA3det-R that amplify band of
1.3 kb (Table S2).

**Data availability**

Strains and plasmids are available upon request. The authors state that all
data necessary for confirming the conclusions presented in the article are
represented fully within the article. Supplemental material available at
FigShare: https://doi.org/10.25387/g3.8796686.

**RESULTS**

**Experimental system**

A diagram showing the approach used to generate tester strains with the
hisG-URA3-hisG cassette is shown in Figure 1A (right side upper
branches). We used the RAD52 locus (Chr6L, left arm of Chr6, coor-
dinates 97,421 to 95,727; see Figure 1B) to determine URA3 loss rates
in Rad52\(^\ast\) strains (RAD52/rad52::hisG-URA3-hisG; wild type in the
context of this study) because it also allows the analysis of rad52 null
mutants (rad52::hisG/rad52::hisG-URA3-hisG), which are refractory to
targeted gene replacement (Ciudad et al. 2004). 5FOA\(^R\) derivatives
from Rad52\(^\ast\) strains can arise through events shown in Figure 2
(Hiraoka et al. 2000; Cauwood et al. 2013). The strategy used to identify
those events is summarized in Fig. S4. SNP-RFLP analysis allowed us
to delimit the genomic region where genetic events responsible for
the RAD52 genotype had occurred. When all SNP markers (snp122,
123 and 132) were homozygous for the same haplotype, the strain was
considered having undergone a chromosome loss event (Figure 2D)
(Legrand et al. 2008; Forche et al. 2011). Truncation of the test chro-
mosome (i.e., the chromosome carrying the URA-Blaster) (Figure 2E)
results in chromosome fragments detectable by pulsed-field gel electro-
phoresis (PFGE) and in hemizygosity of RAD52 and genes between
RAD52 and the left telomere.

**URA3 loss rates in wild type strains and in strains
defective for recombination**

We determined URA3 loss rates and associated recombination mechan-
isms for wild type (RAD52 het, Ura\(^+\)) and for strains deleted for
RAD59, LIG4, RAD52 or RAD51. To further limit the possibility for
single strand annealing to occur, double mutants rad59 rad52 and ligo
erad52 were also analyzed. Importantly, for each genetic background
(except for ligo derivatives), two tester strains carrying the URA-Blaster
on either allele of Chr6 at the RAD52 locus (Chr6A, tester A and Chr6B,
tester B) were analyzed (Figure 1 and Table S1).

For the RAD52 het strains URA3 loss rates for 2 independent assays
was 3.9 \(\times\) 10\(^{-6}\)/cell generation (STD = 6.8 \(\times\) 10\(^{-7}\)) and 2.9 \(\times\) 10\(^{-6}\)/cell
generation (STD = 5.1 \(\times\) 10\(^{-7}\)) respectively (Figures 3 and S5).
No significant differences were observed for a ligo strain (CAGLI01)
carrying the URA-Blaster on Chr6A (Figure 3). For all other single deleted
strains (Table 1), loss rates were on average 2.5- to threefold lower than
those of the wild type equivalent strains, CAGLI1A and CAGL1B, re-
spectively. In contrast, compared to the rad52 single mutant, URA3 loss
rates were higher for rad52 rad59 (5.5-vefold) and rad52 ligo (7.eight-
fold) double mutants (Figure 3) (see Discussion).

**URA3 loss mechanisms in wild type, rad59 and
lig4 strains**

Next, we examined the nature of genetic alterations associated with URA3
loss using CHEF Southernns and SNP-RFLP analysis (Andaluz et al.
2011; Forche et al. 2011). PCR of the RAD52 locus showed that 5FOA\(^R\)
in strains derived from wild type CAGLI1A resulted from URA3 de-
letion (95.5\%, 42/44) and interhomolog recombination (4.5\%, 2/44),
which was similar for CAGL1B 5FOA\(^R\) derivatives (Table 1). Karyotype
analysis did not identify any gross chromosomal rearrangement (GCR)
(Fig. S6) and SNP-RFLP analysis showed homozygosis for snp122 and
snp123 for 5FOA\(^R\) derivatives that resulted from interhomolog recom-
bination and snp132 remained heterozygous (Table S3). This suggests
that crossover/BIR between cen6 and snp123 led to 5FOA\(^R\) in these
derivatives and that both homologs of Chr6 were retained. Taken to-
gether, our data for the RAD52 het strain background show that, irre-
spective of the Chr6 homolog used as the tester allele, the formation of
5FOA\(^R\) derivatives is rarely accompanied by chromosome loss or trun-
cation and a strong bias exists for URA3 deletion vs. interhomolog recombination/other events.

Deletion of RAD59 or LIG4 in wild type strain background did not alter URA3 loss mechanisms. For strains CAGL2A and
CAGL2B (Rad52 het, rad59ΔΔ, Table 1), the URA3 pop-out bias
(88%) was similar to the related wild type strains (Fisher’s exact
test, P = 0.665, tester allele A, and P = 0.314, tester allele B) (Tables
1 and S3). However, three 5FOA\(^R\) derivatives from strain CAGL2B

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showed supernumerary chromosomes (SNC) unrelated to Chr6 (two strains are shown in Fig. S7), suggesting that the absence of Rad59 does not alter frequencies of URA3 pop-out but may cause genetic instability. Similarly, a lig4 strain (Andaluz et al. 2001a; Sinha et al. 2016) carrying the URA-Blaster on Chr6A (CAGL01) did not show GCRs, and all SFOA derivatives resulted from URA3 deletion (Tables 1 and S3) suggesting that microhomology mediated end-joining does not contribute either to the observed URA3 pop-out bias in wild type.

Chromosome loss and truncation are the prevalent mechanisms leading to SFOA derivatives in a rad52 strain background

Because C. albicans strains lacking RAD52 are intrinsically unstable (Andaluz et al. 2011), we first compared two independent, isogenic rad52 mutants (CAGL4A and CAGL4.1A) (Material and Methods, Table S1) that carry the URA-Blaster on Chr6A. CHEF Southern blots indicated that 90% of SFOA derivatives of both strains showed novel SNCs between 815 and 945 kb (Figure 4, Table S2) that hybridized to a COX12 probe (located on Chr6L, Figure 1) suggesting that these were Chr6 truncations. One CAGL4A derivative (CAGL4A-5) acquired a URA3 loss of function mutation (Figure 4, Table S3) and, quite strikingly, one CAGL4.1A derivative (CAGL4.1A-1) showed a wild type genotype except for the absence of URA3 (Figure 4A and C) (see also below).

In contrast to our observations for CAGL4A and CAGL4.1A, chromosome loss was abundant in SFOA derivatives from strain CAGL4B: 6/20 (30%) showed LOH of all three SNP markers (Tables 1 and S3). All other derivatives (14/20; 70%) remained heterozygous for at least one SNP marker. CHEF Southern blots using a COX12 probe showed that 71% (10/14) of them have Chr6-derived SNCs larger than 813 kb (Tables 1 and S3; Figs. S8 and S9). Importantly, as shown for strain CAGL4.1A-1, the remaining four CAGL4B derivatives did not show Chr6 size changes and remained heterozygous for all three SNP markers (Fig. S8B, lanes 9, 10, 12 and 16). In addition, PCR exclusively amplified the hisG repeat suggesting URA3 deletion (Figure 5). Furthermore, and consistent with the heterozygosity of SNP markers, Chr6 bands were brighter and broader on CHEF Southern blots, as expected when two homologs with slightly different sizes are present, compared to less bright, single Chr6 homologs that remain together by the centromere and are maintained together by the centro-mere.

Figure 2 Overview of possible mechanisms leading to inactivation of URA3 in a rad52::hisG-URA3::hisG strain. Homolog A is shown in cyan and homolog B is shown in magenta. The resulting Ura- derivatives (bottom row) are selected on 5-FOA. Viable progeny (only Ura- derivatives can grow on 5-FOA) is indicated with an asterisk. Events can occur in G1 phase of the cell cycle (top row; two homologs) or in G2 (second and third row; both homologs are maintained together by the centro-mere), but only G2 events are shown, as follow: pop-out of URA3 and one copy of the two hisG repeats, which can occur via a single-strand annealing-like mechanism involving spontaneous intrachromatid direct-repeat recombination, intrachromosome or intra-chromatid crossover, or microhomology-mediated end joining (A); unequal sister chromatid exchange (B); inter-homolog recombination including crossover, break-induced replication (BIR) (C), or gene conversion (schematic not shown); ectopic recombination (schematic not shown); chromosome loss (D); chromosome truncation (E); and mutational inactivation of URA3 (F). Green line: Rad52; black line, hisG; gray line, URA3. Note that gene conversion (GC) without crossover at the RAD52 locus in G1 or G2 is also possible, but the absence of heterozygosities between RAD52 and the left telomere prevents its detection.
Overall, and similar to 5FOA<sup>®</sup> rad52 derivatives, URA3 pop-out strains 1) did not show SNCs, 2) remained heterozygous for all three SNPs, 3) conserved both Chr6 homologs (snp132 heterozygous and broader Chr6 bands on PFGE/Southern blots for most of them), and 4) PCR confirmed the presence of an unaltered hisG module and the absence of URA3 (Table S3).

Similarly, the analysis of 5FOA<sup>®</sup> derivatives from strain CAGL6B (lig4 rad52A::hisG/rad52B::URA-Blaster) (Andaluz et al. 2001a) indicated that although most derivatives underwent chromosome loss (17/44; 39%) and chromosome truncation (23/44; 52%) (Tables 1 and S3, Fig. S11), four of them were likely formed by homology-mediated recombination because they satisfied the same four criteria used above for the characterization of rad52 and rad52 rad59 URA3 pop-out derivatives. We conclude that URA3 deletions in rad52 strains may occur in the absence of either Rad59 or Lig4.

Rad52-independent spontaneous recombination result from single strand annealing

In S. cerevisiae, Rad52-independent SSA requires repeats longer than 2 kb (Haber and Hearn 1985; Ozenberger and Roeder 1991; Paques and Haber 1999; Sugawara et al. 2000). However, spontaneous Rad52-independent interhomolog recombination has been reported (Haber and Hearn 1985; Coïc et al. 2008). If this is true for C. albicans, the presence of hisG in a significant fraction of recombinants derived from rad52 strains cannot be attributed exclusively to SSA. Rather, given the allelic configuration of the wild type strains (rad52::hisG/rad52::URA-Blaster), the rad52::hisG allele in 5FOA<sup>®</sup> derivatives could have arisen via conversion of the rad52::hisG-URA3-hisG allele to rad52::hisG (Coïc et al. 2008). To further investigate this, we constructed new rad52 strains carrying the SAT1-cassette which confers resistance to nourseothricin and then recycled SAT1 to generate FRT strains (Figure 1, right side, lower branches and Fig. S1; see also Material and Methods) (Reuss et al. 2004). Two rad52 FRT strains, CAGL4A-FRT and CAGL4B-FRT, showed URA3 loss rates 4- and twofold higher than their hisG isogenic counterparts (Fig. S5) and, more importantly, their 5FOA<sup>®</sup> derivatives contained the rad52::hisG allele (SSA) at frequencies of 25% (5/20) and 10% (2/20), respectively. The remaining events included chromosome loss (45%) and chromosome truncation (45%) for CAGL4B-FRT derivatives and exclusively chromosome truncations (75%) for CAGL4A-FRT derivatives (Tables 1 and S3).

Deletion of either Lig4 or Rad59 in the rad52 FRT background did not alter URA3 loss rates compared to hisG strains or precluded the occurrence of SSA. As shown in Tables 1 and S3, SSA was detectable for two independent rad59 rad52 strains (CAGL5B**-FRT (3/20) and CAGL5B-FRT (5/20)). In addition, chromosome loss and chromosome truncation were also observed (4/20 and 8/20 for CAGL5B**-FRT, and 13/20 and 7/20 for CAGL5B-FRT respectively). Similarly, one SSA-derivative was observed in a lig4 rad52 FRT strain (CAGL6B-FRT), (1/20, 5%) (Tables 1 and S3). Additional events included chromosome loss (55%; 11/20) and chromosome truncation (40%; 8/20) as expected from the tester allele (Tables 1 and S3). Overall, our results unambiguously demonstrate that an SSA-like process led to the generation of hisG from the URA-Blaster in strains lacking RAD52, and that this is also independent of Rad59 and Lig4.

In RAD52 strains, most SSA events are Rad51-independent

URA3 pop-outs/deletions generally result from intra-chromatid recombination either via SSA-like mechanisms, intra-chromatid crossover or unequal sister chromatid exchange. Importantly, whereas both mechanisms

![Figure 3 Determination of URA3 loss rates using fluctuation analysis. Y-axis, Rate of 5FOA resistance/cell/generation. Note: y-axis is a logarithmic scale.](image-url)
require strand invasion and, therefore, are Rad51-dependent. SSA-like events are independent of, if not inhibited by, Rad51 (Ivanov et al. 1996; Jablonovich et al. 1999; Pannunzio et al. 2008; Manthey and Bails 2010; Symington et al. 2014).

To test RAD51 dependency of the observed URA3 pop-outs, we deleted RAD51 in the wild type strain background (CAGL3A and CAGL3B) (Table S1) and found that most CAGL3A 5FOA derivatives still arose via pop-out (35/50) albeit at a decreased frequency (70%) compared to wild type (>90%). The remaining 5FOA derivatives (15/50, 30%) retained RAD52 only. Of these, four strains likely underwent interhomolog recombination (Table 1 and S3). CHEF Southern with a COX12 probe failed to identify Chr6 SNCs among the 5FOA derivatives from URA3 pop-outs (not shown). In contrast, in derivatives that retained RAD52 only, truncations were abundant with SNCs ranging in size from 813 kb to > 850 kb (Figures 6 and S12 and Table S3).

For strain CAGL3B, 76% (38/50) of 5FOA derivatives arose from URA3 pop-outs, whereas only two retained RAD52 and remained heterozygous at all 3 SNPs suggesting that they arose via interhomolog recombination (GC or X0/BIR) near the RAD52 locus (Table 1). In contrast to CAGL3A derivatives, Chr6 truncations were not detected and chromosome loss was significantly more frequent (10/50; 20%) (Tables 3 and S3, Figure 6).

Importantly, CHEF Southern with a COX12 probe revealed novel SNCs with sizes larger than Chr6 for derivatives CAGL3A-2, CAGL3A-18, and CAGL3B-7 (Figure 6, top right). These SNCs also hybridized to a COX12 probe (Figure 6, bottom right), and it is therefore likely that a reciprocal exchange between both RAD52 alleles (gene conversion or crossover) occurred at some step during its generation.

### Table 1 Summary of the events leading to URA3 loss

| Strains | pop-out (URA3 deletion) | Interhomolog recombination | Chromosome loss | Chromosome Truncation | Ectopic Translocation | Mutational Inactivation |
|---------|-------------------------|----------------------------|-----------------|-----------------------|----------------------|------------------------|
| CAGL1A (wt; A; 1) | 100% (24) | — | — | — | — |
| CAGL1A (wt; A; 2) | 90% (18) | 10% (2) | — | — | — |
| CAGL1B (wt; B; 1) | 92% (22) | 8% (2) | — | — | — |
| CAGL1B (wt; B; 2) | 90% (19) | 10% (1) | — | — | — |
| CAGL2A (rad59; A; 1) | 85% (17) | 10% (3) | — | — | — |
| CAGL2A (rad59; A; 2) | 95% (19) | 5% (1) | — | — | — |
| CAGL2B (rad59; B; 1) | 83% (20) | 12% (3) | 5% (1) | — | — |
| CAGL2B (rad59; B; 2) | 90% (17) | 10% (2) | — | — | 5% (1) |
| CAGL3A (rad51, A; 1) | 65% (13) | 10% (3) | — | 20% (4) | — |
| CAGL3A (rad51, A; 2) | 73% (22) | 3% (1) | — | 17% (5) | 7% (2) |
| CAGL3B (rad51, B; 1) | 85% (17) | 5% (1) | — | 15% (2) | — |
| CAGL3B (rad51, B; 2) | 67% (20) | 3% (1) | — | 27% (8) | 3% (1) |
| CAGL4A (rad52, A) | — | — | 90% (9) | — | 10% (1) |
| CAGL4A (rad52, A) | 12.5% (1) | — | — | 87.5% (7) | — |
| CAGL4B (rad52, B) | 20% (4) | — | 30% (6) | 50% (10) | — |
| CAGL5A (rad59 | 10% (1) | — | — | 90% (9) | — |
| CAGL5A (rad59 | 35% (7) | — | — | 65% (13) | — |
| CAGL5B (rad59 | — | — | 11% (1) | 67% (6) | — | 22% (2) |
| CAGL5B (rad59 | 5% (2) | — | 15% (3) | 75% (15) | — |
| CAGL6B (lig4 rad52, B; 1) | 4% (1) | — | 46% (11) | 50% (12) | — |
| CAGL6B (lig4 rad52, B; 2) | 10% (3) | — | 30% (6) | 55% (11) | — | 5% (1) |
| CAGL71 (lig4; A; 1) | 100% (20) | — | — | — | — |
| CAGLAA-FRT | 25% (5) | — | — | — | — |
| CAGLBB-FRT | 10% (2) | — | 55% (11) | 45% (9) | — |
| CAGLBB-FRT | 15% (3) | — | 20% (4) | 65% (13) | — |
| CAGLBB-FRT | 25% (5) | — | 40% (8) | 35% (7) | — |
| CAGLBB-FRT | 5% (1) | — | 55% (11) | 40% (8) | — |
| CAGL72 | 76% (38) | 22% (11) | — | — | 2% (1) |
| CAGL72 | 81% (30) | 19% (7) | — | — | — |

For each strain (except CAGL27 and CAGL28) both genotype and test allele (A or B) are indicated. 1 and 2 refers to independent experiments. The number of derivatives analyzed in each experiment is shown in parenthesis. Genetic events are indicated at the top. For strains CAGL1, CAGL2 and CAGL3, 5FOA derivatives resulting from URA3 pop-out conserved the wild type RAD52 allele and the disrupted rad52::hisG-URA3-hisG allele had been processed to rad52::hisG. Derivatives resulting from IHR carried only the wild type RAD52 allele (2 copies). CL and CT were further confirmed by SNP RFLP analysis and CHEF Southern. nd-not determined. 5FOA derivatives from FRT strains were screened for SSA, SNPs GRCs. Strain CAGL5B-FRT was intended to be CAGL5A-FRT (tester A) since it was derived from CAGL2A but behaved as if carrying RAD52B as the test allele. It is likely that a reciprocal exchange between both RAD52 alleles (gene conversion or crossover) occurred at some step during its generation.
decreased the number of the URA3 pop-outs independent of the allele ($P = 0.0012$, tester allele A; $P = 0.0337$, tester allele B). An interesting consequence of this observation is that, while most pop-out derivatives in the wild type background were generated by SSA, a few may have formed via intra-chromosome crossovers involving sister chromatid exchanges. Furthermore, the absence of Rad51 did not abolish interhomolog recombination since it was still observed among the RAD52 derivatives.

**The majority of SNCs in rad51 5FOA\textsuperscript{R} derivatives are formed by Chr6 truncation followed by telomere addition**

To determine how SNCs larger than wild type Chr6 arose, we tested the possibility that the presence of hisG on other chromosomes could serve as translocation hotspot leading to ectopic translocation and the formation of larger chimeric chromosomes. For example, the size of one of the two reciprocal translocation products involving the hisG repeats of rad52::hisG (Chr6) and rad51::hisG (ChrR, at ~485 kb) would be ~1.4 Mb (900 kb from Chr6 plus 485 kb from ChrR), which is close to the size calculated for the ectopic translocation chromosomal bands of 5FOA\textsuperscript{R} derivatives CAGL3A-2, CAGL3A-18, and CAGL3B-7. However, while primers flanking the RAD52 ORF or the RAD51 ORF amplified the expected fragments, PCRs with mixed primer pairs did not amplify any products suggesting that ectopic translocations did not involve hisG repeats. In addition, CHEF Southern with a hisG probe only hybridized to ChrR in these three strains (Fig. S12, suggesting that the hisG fragment on Chr6 had been lost. Importantly, an HD1 probe from ChrRL (at ~450 kb) failed to co-hybridize with the COX12/CEN6 containing SNCs but hybridized to a novel band of ~1.4 Mb in CAGL3A-23 (Figure 6, bottom left), suggesting that this SNC was generated either by an internal deletion on ChrR or by a translocation involving ChrRL and a centric fragment of one of the smaller chromosomes (Chr5, 6, or 7) (see Figure 6, bottom right). Consistent with either possibility, this SNC, although faintly, hybridized to the hisG probe (Fig. S12), suggesting that it could carry sequences of the rad51::hisG allele.

**RAD52 dosage does not affect URA3 loss**

The wild type strains used in the above experiments (RAD52::hisG-URA3-hisG) carry a single copy of RAD52. To investigate whether RAD52 dosage influences the rate and/or distribution
of events responsible for the Ura- (5FOA²) phenotype, we analyzed derivatives of strain CAGL27 (SHE9/she9::URA-Blaster) (Fig. S2), which carries two RAD52 alleles at its native locus (Materials and Methods) We found that the 5FOA² rate (1.02 x 10⁻⁵ events/cell generation) was on average threefold higher than that calculated for CAGL1A/CAGL1B strains (Figure 3). PCR and SNP marker analysis of 50 independent 5FOA² derivatives showed that 11 (22%) were formed by interhomolog recombination (gene conversion/crossover/ BIR), one (2%) by inactivation of URA3, and 38 (76%) by URA3 pop-outs (Table 1). Importantly, for strain CAGL28, which has a single copy of RAD52, both the 5FOA² rate at the SHE9 locus (1.2 x 10⁻⁵ events/cell generation) (Figure 3) and the distribution of events (81% deletions and 19% interhomolog crossover) were similar to strain CAGL27 (Table 1). Therefore, the RAD52 dosage did not seem to affect the SSA-like bias significantly.

DISCUSSION
In the present study, we aimed to study the mechanisms involved in direct repeat recombination in C. albicans wild type strains and recombination mutants. We took advantage of the URA-Blaster (inserted at the RAD52 locus on Chr6) to determine rates of direct-repeat recombination measured as URA3 loss (resistance to 5FOA), and we used SNP-RFLP and CHEF-Southern analyses to determine the underlying mechanisms. We found that for the wild type strain (CAI4), URA3 pop-outs were the major events responsible for 5FOA² whereas other events identified here as interhomolog recombination and URA3 mutational inactivation were much less frequent and independent of the Chr6 allele examined. Importantly, interhomolog recombination resulted in very long LOH tracts in about 5–10% of 5FOA² derivatives, most of them leading to homozygosis of all SNP markers, which is consistent with BIR or reciprocal crossovers (Forsche et al. 2011; Cauwood et al. 2013; Symington et al. 2014). It is worth noting that the major repeat sequence on Chr6 is located between snp123 marker and cen6 (Figure 1), the region where most interhomolog recombination occurred, suggesting that it could act as a recombination hotspot in wild type cells (Lephart and Magee 2006; Marton et al. 2019). The possibility that these strains exhibit phenotypes attributable to off-target effects calls for an exhaustive characterization of C. albicans genetically engineered strains involving recombination as recently demonstrated for CAI4 (Ciudad et al. 2016).

In the absence of recombination proteins Rad51 or Rad52 chromosome loss and chromosome truncations were frequent, which supports the existence of selective pressure to maintain a complete Chr6A homolog; its loss likely may result in cell death. This conclusion is consistent with previous results indicating that only one homolog of several chromosomes can be lost or is preferentially lost (Andaluz et al. 2011; Hickman et al. 2013). As a diploid, C. albicans may allow the generation of high levels of heterozygosity including the appearance and persistence of recessive lethal alleles of one or more essential genes on one homolog, as was recently shown for Chr4 and Chr7 (Feri et al. 2016; Marton et al. 2019). Therefore, the nature and relative frequency of events responsible for the loss of URA3 may depend significantly on the homolog used as tester chromosome.

Rates of URA3 loss in mutants with defective homologous recombination
We found little variation in URA3 loss rates for single mutants (including rad52) compared to wild type. This is in contrast with the significant decrease in 5-FOA² frequency exhibited by haploid S. cerevisiae rad52 and, to a lesser extent rad59, in a similar assay using URA3 flanked by 2.4 kb-long repeats (Halas et al. 2016). Under these conditions, loss of URA3 via SSA is drastically decreased (rad52) and other mechanisms such as chromosome loss and chromosome truncation may be detrimental. By contrast, a diploid rad52 strain has the potential to become Ura⁻ by chromosome loss and chromosome truncation. This is particularly true for C. albicans, whose genome plasticity is well documented (Rustchenko 2007; Selmecki et al. 2009; Forche 2014). Some variation between the URA3 loss rates of the several strains may also derive from the diploid state of C. albicans. For instance, in wild type S. cerevisiae variation in URA3 loss rates from the URA-Blaster inserted at five different loci was intrinsically greater in diploids compared to haploids (Cauwood et al. 2013). The validity of our assay is further...
supported by the observation that URA3 loss rates were locus- and dosage-independent. The significant increase in URA3 loss rate in rad52 rad59 and rad52 lig4 double mutants compared to rad52 single mutants (5.5 and 7.8-fold, respectively) may simply suggest that Rad59 and Lig4 are suppressing the formation of lesions that form in a rad52 background, most likely because they are repairing these lesions. However, regardless of the mechanism(s) involved, it may stem from the additive effect on genetic instability caused by the lack of more than one gene.

In Rad52+ cells, URA3 pop-outs are Rad59-independent and occur through an SSA-Like mechanism

The absence of Rad59 did not alter the frequency of SSA in C. albicans. This result contrasts with previous work in S. cerevisiae showing that depletion of Rad59 significantly decreased the formation of the SSA product and the number of survivors regardless of repeat length (Sugawara and Haber 1992; Sugawara et al. 2000). An important difference between both systems is that the S. cerevisiae study (Sugawara et al. 2000) used a haploid strain and created a DSB between the repeats whereas we have determined spontaneous recombination in a diploid cell. However, other studies in diploid S. cerevisiae strains have found that Rad59 is also required for spontaneous SSA-like events of short repeats (Halas et al. 2016). It will be interesting to determine whether direct repeats shorter than hisG (1.1 kb) would affect URA3 loss in the absence of CaRad59.

It should be noted that, unlike wild type, the rad59:hisG alleles (Chr4) in rad59 strains could represent potential sites for ectopic translocation involving sequences of the rad52:hisG-URA3-hisG allele (Chr6). We think this is unlikely because translocations involving Chr6 were not observed among the 84 5FOA® derivatives in the rad59ΔΔ strain background. However, it is possible that hisG-mediated ectopic translocations may occur at rates below the limit of detection. In S. cerevisiae, for example, spontaneous ectopic recombination between Ty1 interspersed direct repeats occurred at a much lower rate (10⁻³/cell generation, respectively), which is below the threshold of detection in the system used here (Chan and Kolodner 2011).
Deletion of CaRAD51 does not abolish interhomolog recombination, reduces SSA frequency, and induces ectopic translocation, chromosome loss and chromosome truncation

In *S. cerevisiae*, SSA is Rad51-independent whereas crossovers are dependent on Rad51. We found that depletion of Rad51 caused a statistically significant drop in the rates of URA3 pop-outs. This is in striking contrast to a reported increase in SSA between direct repeats in rad51 null mutants of haploid *S. cerevisiae* (McDonald and Rothstein 1994; Bai and Symington 1996; Ivanov et al. 1996; Jablonovich et al. 1999; Sugawara et al. 2000; Pannunzio et al. 2010; Halas et al. 2016), in rad51 loss-of-function mutants in mammalian cells (Stark et al. 2004), and for Rad51 inhibition of Rad52-mediated annealing of complementary ssDNA in vitro (Wu et al. 2008). The decreased frequency of SSA in the absence of Rad51 suggests that although most URA3 pop-outs in wild type *C. albicans* were due to SSA, a few likely resulted from intra-chromatid crossover. Differences in SSA requirements between both yeasts might arise from the assay conditions (spontaneous in *C. albicans* vs. mostly DSB-induced in *S. cerevisiae*), the ploidy of the strains analyzed, or a differential regulation of SSA/intra-chromatid crossovers that could have evolved in response to the higher number of repeats in *C. albicans* or other species-specific traits.

A second interesting finding was that depletion of Rad51 did not abolish interhomolog recombination, which could still be caused by Rad51-independent BIR (VanHulle et al. 2007) or, more likely, by true crossover/BIR catalyzed by the Rad51-paralog DLH1 (Figure 7). DLH1 is the ortholog of the meiotic recombinase DMC1 that mediates strand invasion in *S. cerevisiae* meiotic cells (Bishop et al. 1992; Diener and Fink 1996). It is clear, however, that an important fraction of the events requiring strand invasion (inter-homolog, inter-sister chromatid, or intra-chromatid crossovers) initiated in the absence of Rad51 are defective and channeled toward ectopic translocation, chromosome loss and chromosome truncation (Table 1, Figure 7). Importantly, whereas ectopic translocations were completely absent in the presence of Rad51, they occurred at rates of $1.5 \times 10^{-7}$ events/cell generation in rad51 strains and likely involved endogenous homologous sequences of another chromosome, but not hisG repeats (Figure 6), further supporting the idea that the latter are not hotspots for translocation.

We have previously shown that centric fragments of truncated chromosomes observed in rad52 strains of *C. albicans* are maintained when sealed by de novo telomere addition using junction sequences

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**Figure 7** Model for genetic events in *rad51* mutants. Left. Spontaneous (likely DLH1-mediated) recombination (crossover/BIR) between Chr6 homologs in G1 followed by the occurrence of a DSB. Each line accounts for ssDNA. Right. A DSB within the cassette region (fragile site) is followed by resection. Telomere addition at the resected end results in SNC formation (see Discussion). In *rad51* strains ectopic translocation can occur if a complementary ssDNA tailed duplex of another broken and resected chromosome (red lines) is detected using the annealing activity of Rad52. If resection continues and trespasses a threshold (which we have traced to the neighborhood of SNP123) the Chr6 fragment cannot be maintained in the absence of Rad52 (and perhaps Rad51) resulting in chromosome loss (loss of Chr6B) or cell dead (loss of Chr6A).
common to both chromosome and telomere (Andaluz et al. 2011). Therefore, it is likely that some resecting of DSBs occurs before bases complementary to telomere repeats are exposed. Similarly, resection of DSBs can also expose ssDNA tracts complementary to sequences present on a different chromosome (Figure 7). In this scenario the absence of Rad51 would increase the substrate pool for translocation mediated by RAD52/RAD9-dependent SSA, consistent with the absence of translocations in rad51 mutants. We conclude that Rad51 is a strong suppressor of spontaneous ectopic translocation in C. albicans. This is consistent with findings in rad51 haploid S. cerevisiae where spontaneous Ty1-mediated GCR rates were increased sevenfold (Chan and Kolodner 2011) and with the spontaneous ectopic translocation frequency between 300 bp of identical sequence in diploids (Pannunzio et al. 2008; Manthey and Bailis 2010). In the latter case, the rate of translocation for Scrad51 strains was 1.1 × 10−7/cell generation, which is similar to what we found for Carad51 in our study.

**On the generation of SNCs in the absence of CaRad52**

Our assay did not select for rad52 SFOA8 derivatives carrying a centromeric SNC that retains a functional URA3. Given the size of Chr6 (1032 kb) and the distance between rad52-hisG-URA3-hisG and the left telomere (≈ 95 kb), the maximum expected size for a SNC is 940 kb, which is consistent with the observation that SNCs were always smaller than 945 kb (the size of Chr7). However, there are no constraints for the minimal size of a Chr6 SNC other than retention of cent6, which is only 53 kb away from the right telomere. In fact, a 95 kb centromere fragment was conserved following truncation of Chr6 in vivo (Baum et al. 2006). The large size of SNCs (usually > 813 kb and never smaller than 666 kb), could stem from the inability of rad52 cells to maintain SNCs smaller than 500 - 600 kb (high chromosome loss frequency). In agreement with this, it was shown previously that the only Chr6-derived SNC identified among spontaneous histidine auxotrophs generated by rad52 cells was ≈ 600 kb (Andaluz et al. 2011), which matches the size of the smallest SNCs detected in this study (≈ 630 kb). One explanation for the abundance of large SNCs is the presence of a fragile site between SNP122 and the left telomere whose tendency to break increases during DNA replication in the absence of Rad52 and, to a lesser extent, Rad51. Importantly, in rad52 or rad51 haploid S. cerevisiae, defective fork restart at damaged (methylated) sites results in chromosome breakage and cell death (González-Prieto et al. 2009). We also observed that the initial break at the URA-Blaster would require a minimum of 100 - 120 kb resection which, according to data from S. cerevisiae, could be too long for wild type (Zhu et al. 2008) but not for rad52 strains (Sugawara and Haber 1992). We do not rule out that the C. albicans major repeat sequence also may act as a recombination hotspot (Lephart and Magee 2006; Chibana and Magee 2009; Marton et al. 2019) and become a breakage site in the absence of homologous recombination proteins. However, if that were the case, resulting SNCs would be too small to be maintained in rad52 strains.

In this study we show that although recombination pathways are basically conserved, C. albicans exhibits specific requirements for mitotic recombination that affect expansion and contractions of repeated sequences, including Rad52-independent SSA (for repeat lengths as short as 1.2 kb) and Rad51-independent interhomolog recombination. This opens up the exciting possibility that, in addition to affecting genome structure, specific features of the recombination machinery may have evolved to facilitate variation. Ongoing and future research will be studying the impact of specific stresses on repeat stability and identifying recombination requirements for repeats of reduced length.

**ACKNOWLEDGMENTS**

We are indebted to Andrés Aguilera for allowing us to do experiments in his lab and to Joachim Morschhäuser for providing the SAT1 cassette. A.F. is funded by NIH grant R15AI090633.

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