Phenotypic Consequences of a Spontaneous Loss of Heterozygosity in a Common Laboratory Strain of *Candida albicans*

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ABSTRACT By testing the susceptibility to DNA damaging agents of several *Candida albicans* mutant strains derived from the commonly used laboratory strain, CAI4, we uncovered sensitivity to methyl methanesulfonate (MMS) in CAI4 and its derivatives, but not in CAF2-1. This sensitivity is not a result of *URA3* disruption because the phenotype was not restored after *URA3* reintroduction. Rather, we found that homozygosity of a short region of chromosome 3R (Chr3R), which is naturally heterozygous in the MMS-resistant-related strains CAF4-2 and CAF2-1, confers MMS sensitivity and modulates growth polarization in response to MMS. Furthermore, induction of homozygosity in this region in CAF2-1 or CAF4-2 resulted in MMS sensitivity. We identified 11 genes by SNP/comparative genomic hybridization containing only the *a* alleles in all the MMS-sensitive strains. Four candidate genes, *SNF5*, *POL1*, *orf19.5854.1*, and *MBP1*, were analyzed by generating hemizygous configurations in CAF2-1 and CAF4-2 for each allele of all four genes. Only hemizygous *MBP1a/mbp1b::SAT1-FLIP* strains became MMS sensitive, indicating that *MBP1a* in the homo- or hemizygous state was sufficient to account for the MMS-sensitive phenotype. In yeast, Mbp1 regulates G1/S genes involved in DNA repair. A second region of homozygosity on Chr2L increased MMS sensitivity in CAI4 (Chr3R homozygous) but not CAF4-2 (Chr3R heterozygous). This is the first example of sign epistasis in *C. albicans*.

KEYWORDS *Candida albicans*; LOH; MMS susceptibility; *MBP1*; growth polarization

THE opportunistic fungal pathogen *Candida albicans* is often isolated as a highly heterozygous diploid; the genome of the reference strain SC5314 has 67,500 single nucleotide polymorphisms (SNPs) (Jones *et al.* 2004; Braun *et al.* 2005; Muzzey *et al.* 2013). SNPs found within regulatory regions can affect transcription levels between the alleles (Staib *et al.* 2002). Even synonymous SNPs residing in open reading frames (ORFs) can result in differences in the rate and efficiency of messenger RNA (mRNA) translation since poorly used codons delay protein synthesis. These delays may lead

to misfolding of the nascent protein or formation of mRNA secondary structures (reviewed in Larriba and Calderone 2008). Recent genome-wide analysis of *cis* elements on gene expression showed that allele-specific effects are often due to mRNA levels and/or translation efficiency (Muzzey *et al.* 2014).

While the majority of SNPs reside in intergenic regions, more than half of open reading frames contain one or more SNPs. The vast majority (78%) of these are nonsynonymous, implying that a significant fraction of ORFs encode proteins that differ in one or more amino acids (Jones *et al.* 2004) that may affect crucial properties. Nonconservative amino acid substitutions within an enzyme's catalytic domain could result in an inactive allele (Gómez-Raja *et al.* 2008). However, many SNPs will cause only minor or insignificant alterations in protein properties.

Mitotic recombination or less frequently, chromosome truncation or loss, will reveal deleterious allele that were masked by a functional allele in heterozygous diploid

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organisms like *C. albicans*. These loss-of-heterozygosity (LOH) events can occur over long ranges of the chromosome (LR-LOH) to homozygote many genes and that could cause new genetic interactions that yield unexpected phenotypes (Weinreich *et al.* 2005). LOH in regulatory regions can alter gene expression responses to some environmental conditions (Staib *et al.* 2002).

In animal cells, LR-LOH causes genetic instability, and along with aneuploidy, is associated with human disease and observed in >90% of solid tumors. *C. albicans* exploits these natural occurrences to generate new phenotypes, including but not limited to auxotrophy (Gómez-Raja *et al.* 2008), mating proficiency (Magee and Magee 2000), and antifungal drug resistance (Selmecki *et al.* 2006, 2008; Niimi *et al.* 2010; Sasse *et al.* 2012).

In *C. albicans*, the frequency of spontaneous LOH is 10^{-4} – 10^{-6} (Forche *et al.* 2009b; Lephart and Magee 2006) and increases significantly under stress conditions *in vitro* (Forche *et al.* 2011), *in vivo* (Forche *et al.* 2009b), or during molecular manipulations such as construction of modified laboratory strains (Selmecki *et al.* 2006; Arbour *et al.* 2009; Bouchonville *et al.* 2009; Abbey *et al.* 2011). Importantly, short- or long-range LOH events yield new genotypes and, potentially, new phenotypes that are heritable. Thus, molecular manipulation of strains carries with it the risk of introducing unidentified mutations (*i.e.*, LOH).

CAI4 is a commonly used *ura3ΔΔ* derivative of *C. albicans*-type strain SC5314 (Fonzi and Irwin 1993) that was derived directly from *URA3/ura3Δ* strain CAF2-1. Importantly, when genes involved the nonhomologous end-joining (NHEJ) pathway of DNA repair are deleted in CAI4, we detected two new phenotypes: MMS- and temperature sensitivity. Here, we show that both phenotypes result from genetic alterations unrelated to deletion of NHEJ genes. MMS sensitivity results initially from an LOH on the right arm of chromosome 3 (Chr3R) that occurred during the construction of CAI4. This region contains the *MBP1* ORF and when one of the alleles (allele *a*) is present either in a homozygous or hemizygous state it is sufficient to confer MMS sensitivity. Furthermore, we identified an additional LOH event on Chr2L in some derivatives of CAI4. While the LOH of Chr2L is neutral by itself, it enhances MMS sensitivity when found together with the Chr3R homozygosity and suggests sign epistasis. Our results are consistent with previous findings that genetically manipulated laboratory strains of *C. albicans* contain additional, nontarget genetic alterations that result in unexpected phenotypes. Furthermore, some of these alterations are the underpinnings for genome and protein diversification, and different selection pressures, depending on the environment, will determine the expansion of specific variants (Ford *et al.* 2015).

Materials and Methods

Strains and growth conditions

C. albicans strains used in this study are listed in Supplemental Material, Table S1.

Cells were routinely grown in solid or liquid YPD (2% glucose, 1% yeast extract, 2% bactopectone, 25 μg/ml uridine) unless otherwise specified.

C. albicans transformation and selection of transformants

To generate gene disruptions, parental strains were transformed with a *SAT1*-flipper cassette by electroporation in a MicroPulser Electroporator system (Bio-Rad, Hercules, CA) (Reuss *et al.* 2004). Nourseothricin-resistant (Nou^R) colonies were selected on YPD plates supplemented with 200 μg/ml of nourseothricin. *SAT1* loss was induced by overnight growth in liquid YPM (2% maltose, 1% yeast extract, 2% bactopectone) and nourseothricin-sensitive (Nou^S) derivatives were selected on YPD plates containing 20 μg/ml of nourseothricin.

In order to look at the consequences of long-range LOH, *URA3* was integrated into Uri[−] strains to generate *URA3* heterozygotes (Wilson *et al.* 1999). The *URA3* cassettes were PCR amplified from pGEM-*URA3* using oligonucleotides complementary to the flanking regions of the selected integration positions on Chr2L or Chr3R (Table S2), and Uri⁺ transformants were selected on SC plates lacking uridine (0.7% yeast nitrogen base, 2% glucose). To isolate derivatives that had undergone LOH, Uri⁺ strains were grown in liquid YPD for 16 hr and subsequently 100 μl of the culture was spread onto SC plates containing 0.1% 5-FOA and 25 μg/ml uridine to select for 5-FOA^R colonies. Reintegration of *IRO1-URA3* in CAI4 and CAI4-L was as described previously (Noble and Johnson 2005). *MBP1* integration was generated through transformation of TCS570 with the *ApaI*–*BglI* fragment of pTC47 (Figure 7B) and Nou^R colonies were selected.

Construction of disruption cassettes and gene cloning

Cassettes for disruption of *SNF5*, *POL1*, *orf19.5854.1*, or *MBP1*: Upstream and downstream regions of each ORF were PCR amplified from the genomic DNA of CAF2-1 with oligonucleotides listed in Table S2, and each *ApaI*–*XhoI* (upstream) and *SacII*–*SacI* (downstream) fragment subsequently cloned in the pSFS2A plasmid (provided by J. Morschhäuser) flanking the *SAT1*-flipper cassette. Each disruption cassette was released by digestion with *ApaI* and *SacI* before transformation.

Cloning and integration of *MBP1b* into its own locus: The full *MBP1* ORF flanked by 276 bp and 243 bp of its upstream and downstream regions, respectively, was PCR amplified from the genomic DNA of TCS1070 (*mbp1aΔ::SAT1-FLIP/MBP1b*) using primers *MBP1*–*ApaI* and *MBP1*–*SacI*. The resulting blunt-ended fragment was cloned into plasmid pNIM1 (from which CaGFP had been previously released by incubation with *SalI* and *BglII*) (Park and Morschhäuser 2005) to yield plasmid pTC46. The 243-bp region downstream of *MBP1* was newly amplified from genomic DNA with primers *MBP1*–*SacII* and *MBP1*–*SacI* and cloned by blunt-end ligation in the *MluI* position of pTC46 to generate pTC47. The *MBP1b* integration cassette was released from pTC47 with *ApaI* and *BglII*.

Figure 1 Sensitivity assay to MMS and temperature of *C. albicans* strains derived from strain CAI4-L. (A) Fivefold serial dilutions of both heterozygous and homozygous *ku70* mutants derived from strain CAI4-L were spotted on YPD plates supplemented or not with 0.03% MMS. Plates were incubated at 28°. For temperature sensitivity YPD plates inoculated with the same strains were incubated at 28° and 43°. Images were taken after 40 hr. (B) MMS and temperature sensitivity of two independent CAI4-L derivatives (TCS570 and TCS571) in which *IRO1* and *URA3* had been reintegrated in its own locus.

MMS and temperature sensitivity assays

MMS and temperature sensitivity was determined using a drop test assay. For MMS, 7- μ l aliquots of fivefold serial dilutions from exponential cultures (OD (optical density) $\cong 1$) were spotted on YPD plates containing 0.02% or 0.03% (v/v) MMS and incubated for 40 hr at 28° before being photographed. Screenings for the MMS sensitivity of 5-FOA^R segregants were routinely carried out by spotting 7- μ l aliquots of 25-fold dilutions from exponential cultures ($OD \cong 1$) on YPD plates containing 0.02% MMS. YPD plates lacking MMS were used as control. For temperature sensitivity, aliquots were spotted on YPD plates and incubated at 43° and 28° for 40 hr.

Genotyping of polymorphic loci

Genotyping of the indicated polymorphic loci was carried out by either direct sequencing (locus *SNF5* and *SNP71*) or SNP-RFLP analysis of PCR-amplified DNA. For direct sequencing, DNA was PCR amplified for 30 cycles using the Expand High Fidelity^{PLUS} PCR System (Roche) following the manufacturer's instructions for higher accuracy. PCR product was purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and directly sequenced at DNA sequencing facilities at Universidad de Extremadura. Sequences were assembled, edited, and compared to sequences reported on the *Candida* Genome Database (CGD) using Lasergene software (DNASTAR). Locus *SNF5* was PCR amplified from both CAF2-1 and CAI4 genomic DNAs using a set of primers (Table S2). Most primers used for SNP amplification are described in Forche *et al.* 2009. Heterozygosity in the locus *POL1* was detected by PCR amplification of a 360-bp internal fragment using primers 2021-F and 2380-R (sequences listed in Table S2) followed by digestion with endonuclease *HincII* (allele *a* cut, allele *b* no cut).

SNP/CGH arrays

Genomic DNA was prepared from overnight cultures and labeled with Cy3 or Cy5 thymidine, using Klenow polymerase and added to a solution containing Agilent 10 $\times$ blocking agent and 22.5 ml of Agilent 2 $\times$ hybridization buffer as described in Abbey *et al.* (2011). Each mixture of Cy3- and Cy5-labeled DNA was treated according to Agilent standard protocols with incubation for 24 hr at 65°. After incubation, arrays were washed using wash buffers and acetonitrile solutions per Agilent standard protocols. Microarrays were scanned as 16-bit TIFF images and analyzed using BlueFuse for Microarrays (BlueGnome, Cambridge, UK). Data analysis and visualization were performed as previously described (Abbey *et al.* 2011).

Data availability

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article.

Results

CAI4 and its derivatives are MMS and temperature sensitive relative to CAF2-1

Proper expression of *C. albicans* *URA3* (orf19.1716) is important for virulence and for dimorphic transition (Lay *et al.* 1998; Brand *et al.* 2004; Sharkey *et al.* 2005). As such, phenotypic assays often compare Uri⁺ versions of mutants, which have been constructed in a CAI4 background, to CAF2-1 (*URA3/ura3*). However, these observed phenotypic differences may vary when these mutants are compared to CAI4 (*ura3/ura3*). Here, we illustrate this “anomalous” behavior with mutants defective in NHEJ, such as *ku70* Δ and

Figure 2 Phenotypic and genomic analysis of strains Uri⁻ derived from CAF2-1. (A) MMS- and temperature sensitivities of strain CAF2-1 and its Uri⁻ derivatives CAF4-2, CAF3-1, CAI4, and CAI4-L (Fonzi and Irwin 1993). The assay was carried out as described in Figure 1. (B) Visualization of SNP/CGH array data of the same strains. Regions that were homozygous in the reference strain or were not informative were not colored. Heterozygous regions are gray colored and homozygous pink or blue colored. Of note, there is not univocal correlation between pink and *a* or blue and *b*. For Chr3R, pink is *a*, and for Chr2L, blue is *a*. Unambiguous ascription of LOH regions to *aa* or *bb* alleles according to current GCD assembly 22 required analysis of individual SNPs and is indicated in the text (see also Andaluz *et al.* 2011).

lig4Δ, and phenotypes associated with MMS sensitivity. A heterozygous *ku70Δ/KU70 ura3Δ/URA3* strain (LCD1A) was similarly sensitive to $\geq 0.02\%$ MMS (Figure 1A and Figure S1A) as the homozygous *ku70Δ/ku70Δ ura3Δ/URA3* strain (LCD2A), suggesting that *KU70* is haploinsufficient with respect to MMS sensitivity. Alternatively, the *KU70* allele *a* (the only allele present in strain LCD1A) may be inactive or hypofunctional, whereas allele *b* has wild-type function. To distinguish between these hypotheses, we generated new heterozygous strains and selected two types of transformants: one retaining allele *a* (LCD1C, isogenic to LCD1A) and the other retaining allele *b* (LCD1B). Both of these new heterozygous strains were similarly sensitive to 0.03% MMS (Figure S1A), indicating these alleles of *KU70* are haploinsufficient. Intriguingly, LCD3A, a reconstituted strain carrying both *KU70* alleles, had gene expression levels comparable to that of CAF2-1 (Chico *et al.* 2011), but did not suppress the MMS sensitivity phenotype (not shown), a result difficult to reconcile with haploinsufficiency.

The temperature sensitivity phenotype was similarly inconsistent among these strains. The *ku70Δ/ku70Δ ura3Δ/URA3* strain (LCD2A) was only moderately thermosensitive at 43° but unable to grow at 45°, whereas the wild type (CAF2-1, *ura3Δ/URA3*) grew robustly at both temperatures. Like the *ku70* null strain, heterozygous *ku70Δ/KU70 ura3Δ/URA3* strains LCD1A and 1B were thermosensitive, in an allele-independent manner (Figure 1A and Figure S1A). Reintegration of one or both *KU70* alleles in the null strain

failed to restore the temperature-resistant phenotype (not shown).

The anomalous behavior of *ku70* mutants, was further complicated by the observation that both heterozygous and null *ku70* mutants in a Uri⁻ background (*ura3Δ/ura3Δ*) did not differ in MMS sensitivity from CAI4-L (the CAI4 strain maintained in our laboratory) (Chico *et al.* 2011; Figure 1A and Figure S1B). When we directly compared CAI4-L to CAF2-1 for MMS and temperature phenotypes, we observed that CAI4-L was MMS sensitive and thermosensitive (Figure 1). These perplexing results motivated us to investigate the genetic underpinnings of the MMS sensitivity observed in CAI4-L and its derivative strains.

MMS and temperature sensitivity is independent of *URA3* expression

To determine whether the lack of *URA3* (CAI4-L), or its decreased expression, potentially derived from position effects, accounted for MMS and/or temperature sensitivity, we compared CAI4-L (*ura3Δ/ura3Δ*) to *ku70* null strains that were either Uri⁺ (*ku70Δ::hisG-URA3-hisG/ku70Δ::hisG*) or Uri⁻ (*ku70Δ::hisG/ku70Δ::hisG*) for MMS and temperature sensitivity. Similar sensitivities were observed between the aforementioned strains, all of which were markedly less resistant than CAF2-1 (*ura3Δ/URA3*) (Figure 1A), implicating *URA3* expression effects. While reintegration of *URA3* at its endogenous locus in the CAI4-L background (TCS570 and TCS571) restored thermotolerance similar to that of CAF2-1 at 28°, temperature sensitivity at 43° and MMS sensitivity was

Figure 3 MMS sensitivity of the indicated *Uri*⁻ strains derived from CAI4 (Abbey *et al.* 2011). Assays were carried out as described in Figure 1 using YPD plates supplemented with uridine. Strains CAF2-1 and CAI4-L were used as control.

unchanged (Figure 1B). Similarly, supplementing uridine to the growth media resulted in slight growth improvements at 28° but not at 43° in CAI4-L and its *Uri*⁺ derivatives (TSC570 and TSC571) (not shown). Taken together, these results suggest that the MMS- and temperature-sensitive phenotypes of *ku70* deletants constructed in a CAI4-L background cannot be attributed to the absence or low expression of *URA3*.

We next tested the hypothesis that both phenotypes resulted from some unknown genetic event(s) that occurred during the construction of CAI4 or the propagation of CAI4-L in our laboratory. If this were the case, all CAI4-L derivatives should display MMS- and temperature sensitivity regardless of the ORF targeted for deletion. We performed phenotypic analysis of two additional sets of mutants derived from CAI4-L: one set investigating heterozygous and homozygous disruption of *LIG4* (orf19.5798), a gene also involved in NHEJ, and a second set investigating analogous disruptions in *SHE9/MDM33* (orf19.5796), whose gene function is unrelated to DNA metabolism (Andaluz *et al.* 2001; Messerschmitt *et al.* 2003). Consistent with the hypothesis, we observed sensitive phenotypes similar to those seen in *ku70* disrupted strains in both sets of disruption (data not shown).

LOH of Chr3R occurred during CAI4 construction and confers MMS sensitivity

The seminal paper by Fonzi and Irwin (1993) describes construction of three isogenic *ura3Δ::imm434/ura3Δ::imm434* strains, CAF3-1, CAF4-2, and CAI4, by disruption/conversion of the remaining *URA3* allele in CAF2-1 (*ura3Δ::imm434/URA3*). Importantly, there are differences in MMS and temperature sensitivity among these three *Uri*⁻ strains. CAF3-1 and CAF4-2 were MMS resistant, similar to the parental CAF2-1, whereas CAI4 and CAI4-L were sensitive (Figure 2A). This implies that the genetic alteration respon-

sible for MMS sensitivity occurred only in CAI4, but not in CAF3-1 and CAF4-2. Of note, while CAF3-1 and CAF4-2 are MMS resistant, they are extremely thermosensitive at 43°, indicating that the thermo- and MMS sensitivities are unlinked (Figure 2A).

The three isogenic *Uri*⁻ strains were generated independently and through different genetic manipulations. CAF3-1 was obtained by selection on 5-FOA plates (Fonzi and Irwin 1993), and accordingly 5-FOA^R resulted from a recombination event that included either: (1) a long-range gene conversion or (2) crossover/break-induced replication followed by cosegregation of the two *ura3Δ* markers. Both CAF4-2 and CAI4 were products of a second disruption step at the *URA3* locus. The gene replacement event disrupting the remaining *URA3* allele in CAF4-2 was verified (Fonzi and Irwin 1993), while the molecular mechanism underlying the construction of CAI4 was not reported (Figure S2).

It was recently shown that multiple LOH events occurred and accumulated in the laboratory lineage of *C. albicans* strains (Abbey *et al.* 2011). One relevant LOH is ~64 kb of Chr3R, beginning within 12–15 kb from *CEN3* and including the SNP71 marker, which is detected in some stocks of CAI4 and in all of its derivatives (Abbey *et al.* 2011), including the RM series of strains (Alonso-Monge *et al.* 2003), BWP17 (Wilson *et al.* 1999), and the SN series (Noble and Johnson 2005). Furthermore, RM10 harbors a second long-range LOH on Chr2L. MMS sensitivity was evident in all RM derivatives, as in CAI4 (Figure 3).

SNP/CGH analysis of *Uri*⁻ strains (CAF3-1, CAF4-2, and CAI4) revealed several important insights including: (1) all strains remained diploid; (2) in CAF3-1, homozygosis of a large portion of Chr3L initiated via recombination near *CEN3*; (3) CAI4 resulted from either short-range gene conversion at *URA3* or through gene disruption of the remaining *URA3* ORF but not

Figure 4 Effect of Chr3R LR-LOH induction in the MMS sensitivity of strain CAF4-2. (A) Diagram of Chr3 showing the position of the inserted *URA3* ORF in the CAF4-2 derivatives T6 and T7 as well as the rearrangements of Chr3 and Chr2 in several 5-FOA^R (Uri⁻) segregants (P21, P27, P29, P32, P41, and P42) as deduced from the subsequent SNP-RFLP analysis. (B) MMS sensitivity assay of the same 5-FOA^R segregants. The 7-μl aliquots of 25-fold dilutions from exponentially growing cultures were spotted on YPD plates supplemented or not with 0.02% MMS. Plates were incubated at 28° for 40 hr.

from a long-range recombination event; and (4) no Chr3R LOH events were detected in CAF4-2 and CAF3-1 (Figure 2B). Of note, CAI4-L harbors a second long-range LOH event on Chr2L, similar to that detected in RM10, suggesting that this may be a recombination hotspot. Detailed analysis of individual SNPs in these strains supports the inferred chromosomal perturbations (Figure S2).

Chr3R homozygosity is sufficient to confer MMS sensitivity in CAF4-2

The only feature common between CAI4 and its derivatives is the 65-kb homozygous region on Chr3R, suggesting that it confers MMS sensitivity. To test this hypothesis, we induced homozygosity of the Chr3R region close to *CEN3* in CAF4-2, by first inserting *URA3* in select intergenic regions of Chr3R (see below and Figure 4) and selected for LOH on 5-FOA. The Uri⁻ derivatives obtained were tested for MMS susceptibility and for genetic rearrangements, including the allelic status of SNP44 and *SNF5*. T3, a strain in which *URA3* was inserted

4.5 kb from *CEN3* (between coordinates 831 and 832 kb) was MMS resistant and maintained heterozygosity for SNPs on Chr3L and Chr3R (not shown). However, all the 5-FOA^R segregants of T3 exhibited MMS susceptibility. Not only were all the 5-FOA^R segregants homozygous for the *a* allele of SNP44 (Chr3L) but also for an *a* allele marker within *POL1* (Chr3R), suggesting that the LOH event involved loss of the entire Chr3 *b* homolog.

In order to favor LOH events mediated through recombination over those from whole chromosome loss, we inserted *URA3* ~55 kb from *CEN3* (between coordinates 882,317 and 882,533 kb), and subsequently analyzed the 5-FOA^R segregants from two independent transformants, T6 and T7 (Figure 4 and Figure S3). While the majority of 5-FOA^R segregants resulted in the loss of Chr3b (88% and 95% in T7 and T6, respectively), we isolated several 5-FOA^R segregants that were heterozygous for SNP44, implying that LOH was due to recombination (Figure 4A and Figure S3B). The 5-FOA^R strains P41 and P42 were derived from T6, and P21, P27,

Figure 5 Visualization of SNP/CGH array data of Chr3R LOH-induced strains derived from CAF4-2. (A) SNP/CGH profiles of P strains. Regions that were homozygous in the reference strains or were not informative were not colored. As described in Figure 2, for Chr3R, pink is *a*, and for Chr2L, pale blue is *a*. (B) High-resolution depiction of the allelic state of markers located between *CEN3* and CGD coordinate 910,000. Each strain was analyzed by SNP/CGH array and data visualized as described (Abbey *et al.* 2011) using the *C. albicans* genome sequence assembly 21. Regions that were homozygous in the reference strain CAF4-2 or were not informative were not colored. Heterozygous regions are gray colored. Alleles *aa* are pink colored and alleles *bb* blue colored. There exists a perfect correlation between our allele assignment and that reported in sequence assembly 22. A snapshot of the raw data is shown in Figure S4.

P29, and P32 were derived from T7. These recombinant strains fell into two groups: the first group (P27 and P29) was MMS resistant and maintained both alleles of *SNF5*, whereas the second group (P21, P32, P41, and P42) was MMS sensitive and homozygous for *SNF5a* (Figure 4B and Figure S3A). From these results, we conclude that segmental Chr3R homozygosis, including SNP71 and *SNF5*, confers MMS sensitivity in *C. albicans*.

Candidate Chr3R genes responsible for MMS sensitivity

The Chr3R homozygous region in CAI4 includes 11 ORFs (Figure 5 and Table 1) that are highly polymorphic in SC5314 and its derivatives. MMS-sensitive derivatives of CAF4-2 (P21, P32, and P41) are homozygous for the Chr3R *a* alleles of these 11 ORFs (Figure 4, Figure 5, Figure S4, and Table 1), similar to what we observed in CAI4. The remaining MMS-resistant strains (P27 and P29) were a mixture of heterozy-

gous and homozygous (allele *b*) ORFs/DNA tracks (Figure 4, Figure 5B, Figure S4, and Table 1).

While our data cannot unambiguously identify the pathways that yielded the P strains, we propose the following mechanisms (Lee *et al.* 2009; Lee and Petes 2010; St Charles *et al.* 2012). The allelic configurations in MMS-sensitive strains (P21, P32, and P41) are consistent with interhomolog reciprocal exchange between *orf19.5854.1* and *CEN3*, followed by cosegregation of the chromatids carrying the *a* alleles into the same daughter cell (Figure 6, A and B). The allelic configurations in MMS-resistant strains (P27 and P29) are consistent with reciprocal crossover and gene conversion resulting from a double-strand break (DSB) during G1 near the *URA3* insertion site on the *b* homolog (Figure 6C). Such an event would not affect ORFs proximal to *CEN3*, which remained heterozygous, as noted (Figure 6C and Table 1). *CEN3* distal sequences in all the P strains have more

Table 1 Summary of the allelic state of polymorphic ORFs

SNP	ORF	Strains							
		CAF4-2	CAI4	CAI4-L	P41	P21	P27	P29	P32
858,941	<i>19.5854.1</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Ht	Ht	Hm (a)
861,709	<i>MBP1</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Ht	Ht	Hm (a)
875,199	<i>19.5863</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Ht	Ht	Hm (a)
876,707	<i>URK1</i>	Ht	ND	Hm (a)	Hm (a)	Hm (a)	Ht	ND	Hm (a)
885,477	<i>19.5869</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	ND	Ht	Hm (a)
887,499	<i>CTP1</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Hm (b)	Hm (b)	Hm (a)
890,349	<i>RB2-3</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Hm (b)	Ht	Hm (a)
891,327	<i>SNF5</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Hm (b)	Hm (b)	Hm (a)
895,120	<i>POL1</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Hm (b)	Hm (b)	Hm (a)
896,549	<i>POL1</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Hm (b)	Hm (b)	Hm (a)
899,690	<i>VAM3</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Hm (b)	Ht	Hm (a)
906,235	<i>19.5880</i>	Ht	Hm (a)	Hm (a)	Hm (a)	Hm (a)	Hm (b)	Hm (b)	Hm (a)

ORFs are from strain CAF4-2 and its derivatives generated by induction of homozygosity within Chr3R (see Figure 4), as deduced from the SNP/CGH analysis. In homozygous ORFs, alleles *a* and *b* are indicated. Ht, heterozygote; Hm, homozygote; ND, not determined.

complicated patterns, in which some polymorphisms are detected, interspersed in the homozygous regions (alleles *a* for P21, P32, and P41, and alleles *b* for P27 and P29) and are indicative of complex recombination events or additional mutations (Hicks *et al.* 2010; Deem *et al.* 2011). It should be noted that P21, which exhibited the highest MMS sensitivity, also carries a Chr2L LOH event (Figure 4 and Figure S3C; see below).

Of the 11 ORFs in the homozygosed region found in P21, P32, and P41, only two carry only synonymous SNPs. Therefore, the *a* alleles of one or more of the remaining ORFs are likely to be responsible for the MMS-sensitivity phenotype. Based on their reported GO functions in *Candida* and *Saccharomyces* (<http://www.candidagenome.org/> and <http://www.yeastgenome.org/>), four of these ORFs, *SNF5*, *POL1*, *orf19.5854.1*, and *MBP1*, were selected for further study. *SNF5* (*orf19.5871*) encodes a component of the Swi/Snf chromatin remodeling complex and null mutants display increased MMS sensitivity in *Saccharomyces cerevisiae* (Chai *et al.* 2005) and anomalous biofilm formation and other pleiotropic defects in *C. albicans* (Finkel *et al.* 2012). We found that *SNF5* homozygous deletants in the CAF2-1 background (TCS1038 and TCS1039) were more sensitive to MMS than its parent, but not as sensitive as CAI4 (Figure S5A). In SC5314 and CAF2-1, electrophoretic analysis of *SNF5* PCR products indicated the presence of two bands. However, in CAI4 and CAI4-L only the largest band was detected (see CAF4-2 and CAI4 profiles in Figure S3A) and sequencing of *SNF5* from CAF2-1 revealed eight SNPs relative to *SNF5* from CAI4. The sequence from CAI4 therefore identifies the haplotype of the larger allele (not shown). The length differences in *SNF5* alleles results from a 159-bp indel after nt 227 of the *SNF5* coding sequence; consequently the larger allele encodes 53 additional amino acids (Table 2). This larger *SNF5* allele has not been annotated in the CGD or in the recent assembly of a phased diploid *C. albicans* genome (Muzzey *et al.* 2013).

We next asked if *POL1*, *orf19.5854.1*, and *MBP1* had significantly altered *a* alleles that may confer MMS sensitivity when homozygous. *POL1* (*orf19.5873*) encodes the DNA

polymerase required for replication initiation and carries several nonsynonymous SNPs (CGD; Table 2). The *S. cerevisiae* ortholog of *orf19.5854.1* (*orf19.5854.1*) is *YBT1*, which encodes an ATP-binding ATPase that transports and sequesters Pb into the vacuole (Sousa *et al.* 2015). The *a* allele of *orf19.5854.1* carries a nonsense mutation resulting in a 69 amino acid truncation (Table 2). Vacuolar sequestration of MMS may help cell survival in the presence of this toxicant. Finally, *MBP1* (*orf19.5855*) encodes a transcription factor that, together with Swi6, forms MBF to regulate genes involved in DNA replication and repair during the cell cycle and following MMS treatment in *S. cerevisiae* (Travesa *et al.* 2012, and references therein). In *C. albicans*, *MBP1* has six nonsynonymous SNPs, and null mutants have minor growth defects under normal conditions (Hussein *et al.* 2011). We hypothesized that MMS sensitivity may result from a defective *MBP1* allele.

***MBP1a* is responsible for MMS sensitivity in CAI4**

Hemizygosis of either *SNF5* or its adjacent gene, *POL1*, as well as hemizygosis of both of them together (Figure S5A), did not change MMS sensitivity. Similarly, hemizygosis of *orf19.5854.1* did not affect the MMS sensitivity phenotype (Figure S5B). Thus, MMS sensitivity does not appear to be due to hemi- or homozygosis of any of these three genes.

In contrast, hemizygous *MBP1a* strains were MMS sensitive while hemizygous *MBP1b* strains were not MMS sensitive (Figure 7A). Furthermore, consistent with the idea that the defects in *MBP1a* are responsible for the MMS sensitivity, reintegration of the *MBP1b* allele in a *MBP1a/MBP1a* strain restored MMS resistance (Figure 7B) and MMS-resistant strains, P27 and P29, retained *MBP1* heterozygosity. Thus, at least one of the six nonsynonymous SNPs in *MBP1* results in a hypo- or non-functional protein, a premise consistent with the fact that both *MBP1* alleles are similarly expressed (Muzzey *et al.* 2014).

MMS-induced growth polarization

Genotoxic stress, including hydroxyurea and MMS treatments, can trigger growth polarization of wild-type *C. albicans*,

Figure 6 Proposed mechanisms for the generation of the several P strains (FOA^R segregants) derived from *URA3* transformants T6 and T7 as deduced from the SNP-CGH results. (A) Strain P41: BIR or reciprocal crossover without associated conversion initiated by a G2 DSB between *orf19.5854.1* and *CEN3*, which marks the transition from heterozygous markers to LOH (alleles a). (B) P21 and P32: reciprocal crossover with associated conversion resulting from a G1 DSB in homolog a (red) between *orf19.5854.1* and *CEN3*, which is repaired using homolog b (blue). This would explain the existence of a short region homozygous for homolog b sequences around the CGD coordinate 837,000 in both strains. A BIR event (instead of reciprocal crossover) is also possible (not shown). (C) P27 and P29: Reciprocal crossover with associated conversion resulting from a G1 DSB in homolog b in the *URA3* neighborhood. One chromatid is repaired to yield a reciprocal crossover with conversion and the second one is repaired using gene conversion or BIR. *URA3* is indicated with a square. *orf19.5854.1* and *orf19.5880*, which delimit the CAI4 homozygous region, are indicated with a triangle and a diamond, respectively. The unusual homozygosity of the Chr3R region distal to *orf19.5880* (not included in the diagram) prevents the unambiguous identification of the mechanism responsible for the conversion event.

resulting in elongated cells (Shi *et al.* 2007; Sun *et al.* 2011; Wang *et al.* 2012; Loll-Krippelber *et al.* 2014). To determine if MMS-induced filamentation can be attributed to homozygosity of *MBP1a*, we compared CAF2-1 (*ura3Δ/URA3*) and CAI4-L (*ura3Δ/ura3Δ*) cell morphologies in response to MMS. CAF2-1 grew in chains of elongated cells with lateral branches but no long filaments were observed, whereas CAI4-L grew predominantly as long filaments. Reintegration of a single *URA3* at its endogenous locus (strain TCS570) did not alter its cell morphology (Figure 8). Therefore, the loss of *URA3* is not responsible for the enhanced filamentous growth displayed by CAI4-L in response to MMS. However, replacement of one of the *MBP1a* alleles of TCS570 with *MBP1b* resulted in a strain (TCS1090) that showed a response to MMS comparable to that of SC5314 and CAF2-1 (Figure 8). Therefore, we conclude

that the exacerbated MMS-induced filamentous growth observed in CAI4 and its derivatives is due to homozygosity of *MBP1a*.

Sign epistasis results in increased MMS sensitivity of CAI4-L

We next investigated the role of another LOH region, found on Chr2L in CAI4, RM10 and their derivatives, in MMS susceptibility. There is a slight, but reproducible difference in MMS sensitivity between CAI4-L and CAI4 (Figure 9A). When we compare their SNP/CGH profiles, a homozygous region is evident on Chr2L in CAI4-L, whereas heterozygosity is maintained in CAI4 (Figure 2B) (Abbey *et al.* 2011; Andaluz *et al.* 2011). In CAI4-L, this LOH occurred via an interhomolog recombination event at coordinate 1,656,168 and we

Table 2 Polymorphisms present in the translation products of alleles *a* and *b*

ORF	SNPs/INDELS		
	Allele <i>a</i>	Allele <i>b</i>	Position (protein)
orf19.5854.1 (ScYBT1 ortholog, transporter of the ATP-binding cassette family)	END	Q (Gln)	84
MBP1 (Putative component of the MBF transcription complex involved in G1/S cell-cycle progression)	A (Ala)	V (Val)	16
	P (Pro)	S (Ser)	145
	F (Phe)	S (Ser)	152
	D (Asp)	G (Gly)	261
	K (Lys)	T (Thr)	641
	V (Val)	K (Lys)	768
SNF5 (SWI/SNF chromatin remodeling complex subunit involved in transcriptional regulation)	K (Lys)	T (Thr)	270
	53 amino acid insert ^a		76
POL1 (Putative DNA directed DNA polymerase α)	V (Val)	I (Ile)	474
	V (Val)	I (Ile)	886
	I (Ile)	M (Met)	1468

The *a* and *b* alleles are from the indicated ORFs as deduced from CGD (alignment 22) and the present results. Bolded aminoacids are likely involved in changes in Mbp1 activity.

^a Information from our sequencing data.

hypothesized that Chr2L homozygosity may contribute to the MMS sensitivity phenotype. Supporting this idea, P21 (a CAF4-2-derived segregant with Chr3R LOH) was even more MMS sensitive than CAI4, reaching the susceptibility of that seen in CAI4-L (Figure 4B). SNP analysis revealed that P21 acquired Chr2L segmental homozygosity (Figure S3C).

To further evaluate the role of this Chr2L LOH in MMS sensitivity, we constructed two strains (T3 and T9) with *URA3* insertions within *orf19.3148* (between coordinates 1,383,200 and 1,385,782) in a CAI4 background (Figure 9B). Sixteen 5-FOA^R segregants from both T3 and T9 were SNP typed and tested for MMS sensitivity. The 5-FOA^R segregants heterozygous for the SNP60 and SNP56 markers displayed MMS sensitivity similar to that of CAI4, whereas all segregants homozygous for the SNP60 marker were MMS sensitive to a similar degree as CAI4-L (Figure 9, C and D). Importantly, all MMS-sensitive segregants carried only the SNP60a allele, just like CAI4-L (Forche *et al.* 2008). Since SNP68 on Chr2R remained heterozygous (not shown), it is likely that Chr2L-homozygous/MMS-sensitive strains resulted from CO or BIR occurring between the *CEN2* and *orf19.3148*. This is reminiscent of the events postulated to have occurred in CAI4-L, although we cannot exclude the possibility of a terminal truncation (Figure 9E). By contrast, the MMS-resistant 5-FOA^R segregants were heterozygous for SNP60 (Figure 9, C and D) and SNP68 (not shown) and likely restored *orf19.3148* through a GC event using the other allele as a template.

To test if Chr2L LOH is sufficient to confer MMS sensitivity independently of the Chr3R LOH, we constructed *URA3* insertions in the same region of Chr2 in a CAF4-2 background so that Chr3R remained heterozygous. About 60 5-FOA^R isolates from two independent transformants (20 from T1 and 40 from T24) were tested for MMS susceptibility (Figure S6C). We observed a range of MMS sensitivity with some 5-FOA^R segregants that were more sensitive to MMS and others that were similar to the control. Some 5-FOA^R segre-

gants had acquired the LR-LOH on Chr2L (indicated by SNP56 and SNP60 homozygosity) (Figure S6B) but conserved the CAF4-2 MMS resistance (Figure S6C). This is likely a reflection of the intrinsic genomic instability of *C. albicans*, which has shown to be enhanced by stresses including 5-FOA (Wellington and Rustchenko 2005; Rustchenko 2007; Bouchonville *et al.* 2009; Gerstein and Berman 2015) and from random LOH or mutational events (Larriba and Calderone 2008). We infer that Chr2L homozygosity is neutral in isolation but can significantly enhance MMS sensitivity in conjunction with Chr3R LOH and is the first report of sign epistasis in *C. albicans*.

Discussion

In this study, we identified a MMS-sensitive phenotype associated with a Chr3R LOH event that occurred during the construction of CAI4. Besides, a second LR LOH event on Chr2L present in some CAI4 derivatives exacerbates that phenotype, an indicator of sign epistasis. The high degree of heterozygosity of the *C. albicans* genome, as exemplified by sequencing efforts of SC5314 and WO-1 (Butler *et al.* 2009), can be irreversibly modified by LOH events. LOH occurs spontaneously with 10^{-6} events per cell per generation and is elevated by one to two orders of magnitude when cells are exposed to external stressors or undergo transformation protocols utilizing heat shock (Wellington and Rustchenko 2005; Bouchonville *et al.* 2009; Forche *et al.* 2011). During the construction of CAI4, cells were subject to two independent heat shocks with counterselections each (Fonzi and Irwin 1993) and subsequent derivatives constructed for additional auxotrophic markers were again exposed to such stresses (Alonso-Monge *et al.* 2003; Noble and Johnson 2005). Therefore, it is not surprising that these strains acquired tracts of LOH unrelated to their target regions (Abbey *et al.* 2011). Furthermore, these types of laboratory manipulations can increase the occurrence of aneuploidy (Bouchonville *et al.* 2009) and it has been shown that some

Figure 7 MMS sensitivity of CAI4 and derivatives strains is *MBP1* allele dependent. (A) MMS susceptibility of *MBP1a* or *MBP1b* hemizygous mutants derived from strains CAF2-1, TCS1018 (*POL1a/pol1bΔ*), and TCS1031 (*SNF5a/snf5bΔ*). (B) Effect of *MBP1b* integration in the indicated *MBP1a* homozygous strains. (B.1) Cassette for *MBP1b* integration into its locus. (B.2) Upper row, MMS-sensitivity assay. Lower row, verification of correct integration of *MBP1b* in transformants (NAT^R) TCS1090 and TCS1093 (both derived from strain TCS570) that had reverted the MMS-sensitivity phenotype.

stocks of CAI4 carry trisomies of Chr1 and/or Chr2 (Chen *et al.* 2004; Selmecki *et al.* 2005).

The regions of LOH and aneuploidies occurring in commonly used *C. albicans* strains are easily identifiable by the use of high-resolution SNP/CGH microarrays (Abbey *et al.* 2011). Relevant to this work is a short region of Chr3R nearby *CEN3* that homozygosed during the construction of CAI4 (*ura3Δ/ura3Δ*) from CAF2-1 (*ura3Δ/URA3*). Phenotypic analysis of mutants derived from CAI4 is complicated because *URA3* expression levels affect filamentation and virulence properties (Bain *et al.* 2001; Chen *et al.* 2004; Noble and Johnson 2005; Sharkey *et al.* 2005; Noble *et al.* 2010) as well as replication stress (Poulter 1990). Yet we found that, (1) *URA3* reintegration into its endogenous locus does not affect MMS sensitivity and (2) supplementing YPD with additional uridine did not modify MMS sensitivity (Figure 1).

LOH is an irreversible process and thus LOH-associated phenotypes are found in all descendant strains. In this study, we identified MMS and thermosensitive phenotypes associated with mutants constructed in the CAI4 background. In the case of *KU70* (*orf19.1135*), since it is a key player in NHEJ

(Chico *et al.* 2011), one of the pathways for DSB repair (Critchlow and Jackson 1998; Daley *et al.* 2005), it would not be surprising that *Caku70* null strains are sensitive to high MMS concentrations. Indeed, *S. cerevisiae ku70* mutants are sensitive to MMS (Foster *et al.* 2011); besides, in this background, telomeres become shorter and cause thermosensitivity at 37° (Barnes and Rio 1997). Since *C. albicans* is intrinsically more thermotolerant than *S. cerevisiae*, analysis of the thermosensitivity of *Caku70* null strains required incubation at higher temperatures (42–43°). We found that both phenotypes are not due to the targeted deletions but inherent to CAI4 strain. Therefore, it is critical to recognize that random, nontargeted LOH can occur during strain construction and result in unexpected phenotypes, which often are mistakenly attributed to the target mutation if the appropriate comparisons are not made. A lesson derived from our results is that phenotypic characterization, in particular growth polarization in response to 0.02/0.03% MMS, limited to comparison of the Uri⁺ versions of deletants constructed in the CAI4 strain or in some of its descendants to CAF2-1 or SC5314 strains should be revisited.

Figure 8 MMS-induced filamentous growth in *C. albicans* strains CAF2-1 and CAI4-L derivatives. A YPD overnight culture of exponentially growing cells from the indicated strains was refreshed and adjusted to $OD_{600} = 1$. Following a further incubation for 2 hr at 30° with shaking, one half was suspended in YEPD supplemented or not (control) with 0.02% MMS. After 16 hr at 30° with gentle shaking, samples were photographed (DIC). Strain TCS570 is a CAI4-L derivative in which *URA3* has been reintegrated into its locus. Strain TCS1090 is a TCS570 derivative in which *MBP1b* has substituted one of the two *MBP1a* alleles.

This study further emphasizes the role of mitotic recombination in modifying and/or altering phenotypes and diversifying diploid descendants during clonal reproduction without having to go through a sexual cycle (Mandegar and Otto 2007; Otto and Gerstein 2008). It is possible that we could detect additional traits, beyond MMS and thermo-sensitivity, if tested under appropriate selective conditions. Importantly, the diploid state of *C. albicans* tolerates disruptive mutations in one allele, due to heterozygous masking, similar to that seen in animals and other organisms. Phenotypes caused by the mutant allele will reveal themselves after monosomy or LOH homozygosis for that allele.

The diploid state has been considered a capacitor for evolution (Schoustra *et al.* 2007) through accumulation of recessive mutations, provided they show sign epistasis, *i.e.*, mutations in isolation are either neutral or deleterious, but are advantageous in combination or with the appropriate genetic background (Weinreich *et al.* 2005). In this regard, some CAI4 stocks carry additional regions of LOH, such as CAI4-L, which has Chr2L long-range LOH and no detectable aneuploidy (Andaluz *et al.* 2011). We cannot distinguish whether this long-range LOH was spontaneous or a result of unknown stress. This region of Chr2L homozygosis has been detected in RM10 and SN strains, both of which are derived from CAI4 (Abbey *et al.* 2011), as well as among parasexual progeny (Forche *et al.* 2008). The relatively high frequency at which we detect Chr2L homozygosis may be due to a recombination hot spot on Chr2L and/or due to a selec-

tive advantage to this event under laboratory growth conditions. Here, we found that Chr2L homozygosis was neutral for MMS sensitivity when in isolation, but in combination with Chr3R homozygosis, it increased MMS sensitivity. This work represents a clear example of sign epistasis, which has a proposed important role in evolution (Weinreich *et al.* 2005), but has not previously been reported in *C. albicans*.

Sign epistasis may be more frequently detected in complex traits, like MMS sensitivity, that are influenced by hundreds of genes (Begley *et al.* 2002, 2004; Horton and Wilson 2007). Repair of MMS lesions involves pathways including base excision repair (Boiteux and Jinks-Robertson 2013), double strand break repair (Symington *et al.* 2014), chromatin remodeling (Oum *et al.* 2011) pathways, *etc.* Mutations in these genes will likely result in altered sensitivity to MMS. Furthermore, it is likely that combination of weak alleles across multiple genes can cause MMS sensitivity, despite low or no sensitivity associated with any individual weak allele. Weak alleles present in deletion backgrounds may enhance the phenotype of the deleted gene of interest. In *S. cerevisiae*, a mutant allele of *RAD5*, *rad5-G535R*, was sensitive to MMS (Fan *et al.* 1996). However in response to UV light, another complex trait, the interactions between *RAD5* and *RAD52* alleles are more complex. Strains carrying the *rad5-G535R* allele were more UV sensitive, particularly at high doses compared to wild type; strains with *rad52 rad5-G535R* double mutations were more UV sensitive than either mutation in isolation (Fan *et al.* 1996).

Figure 9 Effect of Chr2L LR-LOH induction in the MMS sensitivity of strain CAI4. (A) Comparison of MMS sensitivities of CAI4-L and CAI4. (B) Diagram of Chr2 showing the position of the inserted *URA3* ORF in transformants T3 and T9. Both transformants were PCR verified for the correct insertion of *URA3*. (C) MMS sensitivity of several *FOA^R* segregants (P strains) derived from transformants T3 and T9. (D) RFLP analysis of SNP60 and SNP56 markers from the indicated strains following digestion of the PCR amplification products with *TaqI* and *EcoRV*, respectively (Forche *et al.* 2009a). (E) Diagram of Chr2 showing the LR-LOH region present in all *FOA^R* segregants exhibiting MMS sensitivity similar to CAI4-L.

Given that long-range LOH results in homozygosity of many genes, it is predicted that there will be a number of altered phenotypes associated with LOH, particularly in highly heterozygous organisms. Regions of homozygosity (ROH) have recently been implicated with complex traits and diseases in human, specifically height (Yang *et al.* 2010), schizophrenia, and late-onset Alzheimer's disease (Nalls *et al.* 2009). *C. albicans*, because it is a highly heterozygous diploid, may be a good model for further studies of this nature. A recently identified long-range LOH in clinical isolates of *C. albicans* has been linked to fluconazole resistance and these types of events were both recurrent and persistent (Ford *et al.* 2015). *ERG11* was included in the region of LOH and in some cases, a driver mutation in *ERG11* (*i.e.*, a persistent mutation for a genotype not found in the progenitor strain) was identified. It is likely that the driver mutation arose first in a heterozygous manner and was homozygosed by a subsequent LOH. However, it was not known if this driver mutation was sufficient to cause fluconazole resistance or if other genes within the region of LOH also contributed to the azole resistance phenotype.

Even in the absence of driver mutations, LOH events can confer new phenotypes, since allelic variation occurs within the heterozygous parental strain. This premise is exemplified in our study. Alleles of *MBP1* differ in activity, and hemizygous *MBP1a* strains are more sensitive to MMS than hemizygous *MBP1b* strains. Importantly, *MBP1a* lacks an N-terminal low complexity region (LCR) in which two Ser residues are mutated to Pro and Phe, respectively (Table 2). Terminal LCRs are enriched for translation and stress-response-related terms (Coletta *et al.* 2010). We suggest that substitution of one or both Ser residues (see *Results*) are likely responsible for differences in Mbp1 activity. In particular, Ser¹⁵² (Mbp1b) resides within a Ser-enriched stretch (148–157) and is potentially phosphorylated (NetPhos 2.0 server).

We rule out the possibility that the MMS phenotype is due to a combination of weak alleles within the region of Chr3R LOH: *MBP1a* homozygosity or hemizygosity is sufficient and the simultaneous homozygosity or hemizygosity of *SNF5* and *POL1*, two other candidate polymorphic genes, had no additional effect. We also considered that MMS sensitivity requires homozygosity and not hemizygosity of *SNF5*, since

some fungal phenotypes need transvection between alleles (Aramayo and Metzenberg 1996). However, MMS resistance was not restored when *SNF5* homozygosity in CAI4 was disrupted.

In *S. cerevisiae*, Mbp1 binds DNA and, together with Swi6, forms the transcription complex MBF to regulate genes involved in DNA replication and repair in G1/S phase; MBF activates these genes during G1 and represses them, with Nrm1, outside of the G1 phase. In response to replication stress, phosphorylation of Nrm1 releases MBF to activate genes involved with DNA repair and replication (Travesa *et al.* 2012). In *S. cerevisiae*, *POL1* is an Mbp1 target gene induced in response to genotoxic stress caused by hydroxyurea, MMS, or camptothecin (Travesa *et al.* 2012). It is currently unknown if this regulatory circuit is conserved in *C. albicans*.

Beyond providing evidence that LOH is associated with novel phenotypes in CAI4 (which raises issues for the lab strains derived from it), we also revealed an important feature of *SNF5* that was not previously annotated in the CGD. Taken together with an earlier study reporting 11 new SNPs in *HIS4* (Gómez-Raja *et al.* 2008), we posit that the number of polymorphisms reported for the *C. albicans* SC5314 genome, including SNPs, indels, and truncated ORFs (Jones *et al.* 2004; Braun *et al.* 2005; van het Hoog *et al.* 2007; Muzzey *et al.* 2013), is a conservative estimate and could be even higher than documented in the current databases.

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Phenotypic Consequences of a Spontaneous Loss of Heterozygosity in a Common Laboratory Strain of *Candida albicans*

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Figure S1

A

B

Fig. S1. MMS- and temperature-sensitivity of ku70 mutants derived from strain CAI4-L. For MMS sensitivity, five-fold serial dilutions of both heterozygous and homozygous Uri+ (A) or Uri- (B) ku70 mutants derived from CAI4-L were spotted on YPD plates supplemented or not with 0.03% MMS and compared to CAF2-1 (A) or CAI4-L (B) strains respectively. Plates were incubated at 28 °C. For temperature sensitivity, YPD plates inoculated with the same strains were incubated at 28 °C and 43 °C. Images were taken after 40 hours.

Figure S2

Fig. S2. Diagram showing possible mechanisms leading to generation of the Uri- strains and strategies used to verify their occurrence. To investigate LOH events, three polymorphic sites were analyzed. Two of them, RFLP-SNP 44 marker which is 90 kb telomere proximal to URA3 and an EcoRI site which is 5 kb centromere proximal to URA3, reside on the left arm of Chr3 (Chr3L). The third one, SNP71 marker, is on Chr3R close to the centromere. Of note, although Chr3R of the SC5314 strain and its derivatives is mostly homozygous, colors for homologs a (red) and b (blue) had been maintained across the whole arm to distinguish both alleles of the several heterozygous ORFs. Both Chr3L polymorphic sites, SNP44 and EcoRI, remained heterozygous in strain CAF2-1 and its derivative CAF4-2. By contrast, they had become homozygous in strain CAF3-1, as expected from its generation through a recombination event which included also the EcoRI site. Both SNP44 marker and the EcoRI site close to the URA3 locus remained heterozygous in strain CAI4, suggesting that this strain was generated by either gene replacement or a local GC event using the other homolog as a template, but not by a CO or BIR event (see Fig. 2B). SNP71 marker remained heterozygous in CAF3-1 and CAF4-2 strains, but had become homozygous in CAI4 (Forche et al. SNP map in CGD and our sequencing data) and its lineage including CAI4-L. Of note, the conversion event that specifically caused SNP71 homozygosity in CAI4 and its derivatives was independent of the event responsible for the *ura3* homozygosity. Not only the EcoRI site remained heterozygous in CAI4, but sequencing of CEN3 region from CAI4-L revealed the presence of at least one SNP (Gomez-Raja and Larriba 2013).

Figure S3

Fig. S3. Genotyping of the indicated strains derived from CAF4-2 following induction of LR-LOH in Chr3R (A): Analysis of the SNF5 length polymorphism. FOAR derivatives exhibiting MMS-sensitivity (P41, P42, P21 and P32) are homozygous for SNF5. (B) RFLP analysis of SNP44 marker (Chr3L). (C): RFLP analysis of SNP56 marker (Chr2L). The restriction enzymes used in this analysis are indicated (Forche et al. 2009a).

Figure S4

Chr3 (Kb from
left telomere)

Fig. S4. Snapshot of Chr3R region of CAI4 and the indicated P strains as deduced from SNP/CGH microarray data.

Figure S5

Fig. S5. MMS-sensitivity of CAF4-2 derivatives hemizygous for SNF5, POL1 and orf19.5854.1. (A): Five-fold serial dilutions from exponentially growing cultures of the indicated *snf5* and *pol1* mutants were spotted on YPD plates plus uridine with and without 0.02%MMS and incubated at 28 °C for 40 hours. Strains CAF2-1 and CAI4 were used as control. (B): Similar analysis of orf19.5854.1 deletion mutants.

Figure S6

A

B

C

Fig. S6. Effect of Chr2L LR-LOH in the MMS-sensitivity of strain CAF4-2. (A): PCR verification of correct URA3 integration in Chr2L (orf19. 3148). LR-LOH was induced in each T1 and T24 Uri⁺ transformants and Uri⁻ segregants isolated on 5FOA plates. (B): Chr2L heterozygosity of the indicated FOAR derivatives was determined by SNP60-RFLP analysis (Forche et al. 2009a). (C): Sensitivity to 0.02% MMS of 20 and 40 FOAR from T1 and T24 respectively. 7 μ l aliquot of 25-fold dilutions from exponentially growing cultures (DO₆₀₀ = 1) were spotted on YPD plates plus uridine with and without MMS and then incubated at 28 $^{\circ}$ C for 40 hours.

Table S1. *C. albicans* strains used in this work

Strain	Parent	Relevant genotype/phenotype	Source or reference
CAF2-1	SC5314	<i>ura3Δ::imm434/URA3</i> <i>iro1Δ::imm434/IRO1</i>	Fonzi and Irwin, 1993
CAI4	CAF2-1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i>	Fonzi and Irwin, 1993
CAI4-L	CAI4	LOH in Chr2L telomere proximal region	Reported in Andaluz <i>et al.</i> , 2011
CAF3-1	CAF2-1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i>	Fonzi and Irwin, 1993
CAF4-2	CAF2-1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i>	Fonzi and Irwin, 1993
TCS570	CAI4-L	<i>ura3Δ::imm434/URA3</i> <i>iro1Δ::imm434/IRO1</i>	This study
TCS571	CAI4-L	<i>ura3Δ::imm434/URA3</i> <i>iro1Δ::imm434/IRO1</i>	This study
TCS576	CAI4	<i>ura3Δ::imm434/URA3</i> <i>iro1Δ::imm434/IRO1</i>	This study
LCD1A	CAI4-L	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i> <i>KU70a/ku70bΔ::hisG-URA3-hisG</i>	Chico <i>et al.</i> , 2011
LCD1A.1	LCD1A	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i> <i>KU70a/ku70bΔ::hisG</i>	Chico <i>et al.</i> , 2011
LCD1B	CAI4-L	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i> <i>ku70aΔ::hisG-URA3-hisG/KU70b</i>	Chico <i>et al.</i> , 2011
LCD1C	CAI4-L	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i> <i>KU70a/ku70bΔ::hisG-URA3-hisG</i>	Chico <i>et al.</i> , 2011
LCD2A	LCD1A.1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i> <i>ku70aΔ::hisG-URA3-hisG/ku70bΔ::hisG</i>	Chico <i>et al.</i> , 2011
LCD2A.1	LCD2A	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i> <i>ku70aΔ::hisG/ku70bΔ::hisG</i>	Chico <i>et al.</i> , 2011
LCD3A	LCD1A.1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i> <i>KU70a/KU70b::URA3-hisG</i>	Chico <i>et al.</i> , 2011
P1 (parasexual progeny)	Tetraploid from RBY16 and CHY477 mating	Trisomy of Chr 4; homozygosis of Chrs 3 & 7; partial homozygosis of Chr 2.	⁽¹⁾ Forche <i>et al.</i> (2008)
CAI4-F2	CAF2-1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i>	⁽¹⁾ Fonzi and Irwin, 1993
CAI4-F3	CAF2-1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>iro1Δ::imm434/iro1Δ::imm434</i>	⁽¹⁾ Fonzi and Irwin, 1993

RM10	RM1	<i>ura3Δ::imm434/ura3Δ::imm434 iro1Δ::imm434/iro1Δ::imm434 HIS1/his1Δ</i>	⁽¹⁾ Alonso-Monge <i>et al.</i> , 2003
RM100#13	RM10	<i>ura3Δ::imm434/ura3Δ::imm434 iro1Δ::imm434/iro1Δ::imm434 his1Δ:: URA3 /his1Δ</i>	⁽¹⁾ Alonso-Monge <i>et al.</i> , 2003
RM1000#6	RM100	<i>ura3Δ::imm434/ura3Δ::imm434 iro1Δ::imm434/iro1Δ::imm434 his1Δ/his1Δ</i>	⁽¹⁾ Alonso-Monge <i>et al.</i> , 2003
RM1000#2	RM100	<i>ura3Δ::imm434/ura3Δ::imm434 iro1Δ::imm434/iro1Δ::imm434 his1Δ/his1Δ</i>	⁽¹⁾ Navarro-García <i>et al.</i> , 2003
BWP17	RM1000#1	<i>ura3Δ::imm434/ura3Δ::imm434 iro1Δ::imm434/iro1Δ::imm434 his1Δ/his1Δ arg4Δ/arg4Δ</i>	⁽¹⁾ Wilson <i>et al.</i> , 1999
SN76	RM1000#2	<i>ura3Δ::imm434/ura3Δ::imm434 iro1Δ::imm434/iro1Δ::imm434 his1Δ/his1Δ arg4Δ/arg4Δ</i>	⁽¹⁾ Noble and Johnson., 2005
TCS1007	CAF2-1	<i>SNF5a/snf5bΔ::SAT1-FLIP</i>	This study
TCS1008	CAF2-1	<i>SNF5a/snf5bΔ::SAT1-FLIP</i>	This study
TCS1010	CAF2-1	<i>snf5aΔ::SAT1-FLIP/SNF5b</i>	This study
TCS1011	CAF2-1	<i>snf5aΔ::SAT1-FLIP/SNF5b</i>	This study
TCS1013	CAF2-1	<i>pol1aΔ::SAT1-FLIP/POL1b</i>	This study
TCS1014	CAF2-1	<i>pol1aΔ::SAT1-FLIP/POL1b</i>	This study
TCS1016	CAF2-1	<i>POL1a/pol1bΔ::SAT1-FLIP</i>	This study
TCS1017	CAF2-1	<i>POL1a/pol1bΔ::SAT1-FLIP</i>	This study
TCS1018	TCS1016	<i>POL1a /pol1bΔ::FRT</i>	This study
TCS1019	TCS1017	<i>POL1a /pol1bΔ::FRT</i>	This study
TCS1020	TCS1018	<i>POL1a /pol1bΔ::FRT SNF5a/snf5bΔ::SAT1-FLIP</i>	This study
TCS1021	TCS1019	<i>POL1a /pol1bΔ::FRT SNF5a/snf5bΔ::SAT1-FLIP</i>	This study
TCS1022	TCS1019	<i>POL1a /pol1bΔ::FRT SNF5a/snf5bΔ::SAT1-FLIP</i>	This study
TCS1023	TCS1018	<i>POL1a /pol1bΔ::FRT snf5aΔ::SAT1-FLIP/SNF5b</i>	This study
TCS1024	TCS1019	<i>POL1a /pol1bΔ::FRT snf5aΔ::SAT1-FLIP/SNF5b</i>	This study
TCS1031	TCS1007	<i>SNF5a/snf5bΔ::FRT</i>	This study
TCS1038	TCS1031	<i>snf5aΔ::SAT1-FLIP/snf5bΔ::FRT</i>	This study
TCS1039	TCS1031	<i>snf5aΔ::SAT1-FLIP/snf5bΔ::FRT</i>	This study
TCS1040	CAI4	<i>snf5aΔ::SAT1-FLIP/SNF5a</i>	This study
TCS1041	CAI4	<i>snf5aΔ::SAT1-FLIP/SNF5a</i>	This study
TCS1042	CAI4	<i>snf5aΔ::SAT1-FLIP/SNF5a</i>	This study
TCS1043	CAI4	<i>snf5aΔ::SAT1-FLIP/SNF5a</i>	This study
TCS1060	CAF4-2	<i>orf19.5854.1aΔ:: SAT1- FLIP/orf19.5854.1b</i>	This study
TCS1061	CAF4-2	<i>orf19.5854.1a/orf19.5854.1bΔ::SAT1- FLIP</i>	This study

TCS1062	CAF4-2	<i>orf19.5854.1aΔ:: SAT1-FLIP/orf19.5854.1b</i>	This study
TCS1063	CAF4-2	<i>orf19.5854.1aΔ:: SAT1-FLIP/orf19.5854.1b</i>	This study
TCS1070	CAF2-1	<i>mbp1aΔ::SAT1-FLIP/MBP1b</i>	This study
TCS1071	CAF2-1	<i>MBP1a/mbp1bΔ::SAT1-FLIP</i>	This study
TCS1072	TCS1018	<i>POL1a /pol1bΔ::FRT</i> <i>mbp1aΔ::SAT1-FLIP/MBP1b</i>	This study
TCS1081	TCS1018	<i>POL1a /pol1bΔ::FRT</i> <i>MBP1a/mbp1bΔ::SAT1-FLIP</i>	This study
TCS1074	TCS1031	<i>SNF5a/snf5bΔ::FRT</i> <i>MBP1a/mbp1bΔ::SAT1-FLIP</i>	This study
TCS1085	TCS1031	<i>SNF5a/snf5bΔ::FRT</i> <i>mbp1aΔ::SAT1-FLIP/MBP1b</i>	This study
TCS1088	TCS570	<i>MBP1a/MBP1a</i>	This study
TCS1089	TCS570	<i>MBP1a/MBP1a</i>	This study
TCS1090	TCS570	<i>MBP1a/MBP1b::SAT1-FLIP</i>	This study
TCS1091	TCS570	<i>MBP1a/MBP1a</i>	This study
TCS1092	TCS570	<i>MBP1a/MBP1a</i>	This study
TCS1093	TCS570	<i>MBP1a/MBP1b::SAT1-FLIP</i>	This study
TCS1094	TCS576	<i>MBP1a/MBP1a</i>	This study
TCS1095	TCS576	<i>MBP1a/MBP1a</i>	This study
TCS1096	TCS576	<i>MBP1a/MBP1a</i>	This study
TCS1097	TCS576	<i>MBP1a/MBP1a</i>	This study
TCS1098	TCS576	<i>MBP1a/MBP1a</i>	This study

⁽¹⁾ Strains from J. Berman stock

Table S2: Oligonucleotides used in this work

Primer	Primer sequence	Usage
URA3 Left Flank	GATGTTCTTGAAGCGTAAAAC	<i>URA3</i> and <i>IRO1</i> restoration
URA3 Right Flank	CACCGTCGATAGTTTTACGG	<i>URA3</i> and <i>IRO1</i> restoration
SNF5-11-F	TGACGTCAACAATGAGTT	<i>SNF5</i> sequencing/PCR checking of <i>SNF5</i> allele
SNF5+381-F	TCAACCCATTTCATGGAGC	<i>SNF5</i> sequencing
SNF5+794-F	TGATTAGAAAGGATAAGG	<i>SNF5</i> sequencing
SNF5+1182-F	AAAAGTGGAAGATTTTCAC	<i>SNF5</i> sequencing
SNF5+1581-F	TTTATTGCCACCTTTAAG	<i>SNF5</i> sequencing
SNF5+1981-F	AATCCAGATTATAGACCG	<i>SNF5</i> sequencing
SNF5+2191-R	ACAACCATAACCCTGTGA	<i>SNF5</i> sequencing
SNF5+1791-R	AGAACCCTGTTGCTGATC	<i>SNF5</i> sequencing
SNF5+1388-R	ATATCCCATTCAAATTGG	<i>SNF5</i> sequencing
SNF5+991-R	CATTGCCATAACCTTGAT	<i>SNF5</i> sequencing
SNF5+590-R	GGTACCCCTGTAGCCAAT	<i>SNF5</i> sequencing/PCR checking of <i>SNF5</i> allele
SNF5+191-R	TACTGGTTCATTACTTCT	<i>SNF5</i> sequencing
SNF5-22-R	ATCGTATCTATGTATACC	<i>SNF5</i> sequencing
SNF5-F- <i>Apa</i> I	GCgggcccGCTTGTCTTGTC	PCR amplification of a <i>SNF5</i>
SNF5-R- <i>Xho</i> I	GCATctcgagGTTGACGTCAAGAC	upstream fragment
SNF5-F- <i>Sac</i> II	GCccgaggGTATAATTCATG	PCR amplification of a <i>SNF5</i>
SNF5-R- <i>Sac</i> I	GTCgagctcGTTGATTGTAAAGC	downstream fragment

POL1-479-F	CAAAC TATTTCCCTGAAA	<i>SAT1</i> loss in strains TCS1018 y TCS1019
POL1+2021-F	GTCTTCTCATTCGTGATG	PCR checking for <i>POL1</i> heterozygosity (SNP-RFLP with <i>HincII</i>)
POL1+4021-F	TTGGTTTGTGATGACCCT	<i>SAT1</i> loss in strain TCS1031
POL1+4521-F	GCAAAC TTTACTTATCGT	PCR checking of strains TCS1040/1041/1042/1043
POL1+4880-R	TCTTGAAATTGAGGATTG	<i>SAT1</i> loss in strains TCS1018 y TCS1019
POL1+2380-R	CATGTAACAAAATGTATT	PCR checking for <i>POL1</i> heterozygosity (SNP-RFLP with <i>HincII</i>)
POL1int-F	CCAGTCTATCTTTGATGTTG	<i>POL1</i> disruption checking
POL1-F- <i>ApaI</i>	GCgggcccCACGTCCCATTC	PCR amplification of a <i>POL1</i> upstream fragment
POL1-R- <i>XhoI</i>	GCA TctcgagATCTGGACAGCATAG	PCR amplification of a <i>POL1</i> downstream fragment
POL1-F- <i>SacII</i>	GCcgcggCCTTGGTGGAT	PCR amplification of a <i>POL1</i> downstream fragment
POL1-R- <i>SacI</i>	GCGgagctcCTACTCTTAGTTCC	PCR amplification of a <i>POL1</i> downstream fragment
MBP1-F- <i>ApaI</i>	TAgggcccAAAAATAGAACGA	PCR amplification of a <i>MBP1</i> upstream fragment
MBP1-R- <i>XhoI</i>	GCA TctcgagGTTTAGAAAGTG	PCR amplification of a <i>MBP1</i> downstream fragment
MBP1-F- <i>SacII</i>	TAccgcggATGCTATGGAAG	<i>MBP1</i> disruption checking
MBP1-R- <i>SacI</i>	GTCgagctcTATTGCTCATAGAC	PCR checking for <i>MBP1</i> heterozygosity (SNP-RFLP with <i>EcoRI</i>)
MBP1-R	GCAATTTAGACACAGTTATTACTG	PCR amplification of a <i>orf19.5854.1</i> upstream fragment
MBP1-1F	GTAGTCGACCACATCAATAACAC	PCR amplification of a <i>orf19.5854.1</i> downstream fragment
MBP1-1R	CGTAAGATCTCATTATGTATATTTAGTAC	<i>orf19.5854.1</i> disruption checking
orf19.5854.1-F- <i>ApaI</i>	TAgggcccATTATTTTGTGAGC	PCR checking for gene disruption with SAT1-flipper cassette
orf19.5854.1-R- <i>XhoI</i>	GCA TctcgagAATTGAACCAG	
orf19.5854.1-F- <i>SacII</i>	TAccgcggTAGTCTTGGATG	
orf19.5854.1-R- <i>SacI</i>	GTCgagctcGAATCATTGGTC	
orf19.5854.1-R	CAATGGATGAAATTGAAACTTCG	
SAT1flip-F	CTGGTACTGGTTCTCGGG	
SAT1flip-R	CCCGAGAACCAGTACCAG	
19.3148-5DR	GAGTCTACACCAAGCACCTAATATTTGTTCATACAAACCCCAAC TCAACTCAACTCAACATTACAACA ACAACAACAACAATAAAGCgttttccagtcacgacgtt	<i>URA3</i> integration in Chr2L for LOH induction
19.3148-3DR	CGTTTATAATTATGAAATCAAATAAAAATATTTATTAAACCATGTCTC CATAACCCCTCCCATATAGTCTAC CTCTTTCTATCTTATgtggaattgtgagcggtata	<i>URA3</i> integration in Chr2L for LOH induction
Chr3RA-5DR	GCTTATCAACAAAACAATAGAGTACATCATTTTCATTCAAAGTTGGAAAAC TTTGTTCAATTTAGATCTTGTC AATTGAGCgttttccagtcacgacgtt	<i>URA3</i> integration in Chr3R (between <i>AAP1</i> and <i>CTN3</i>) for LOH induction

Chr3RA-3DR	CACGTGACTCAAACAAAAGTTAGT GTATCACCCTACCATGCAAACAA TAGAGCTGTTTACAAGATTAGAGA TTAATTCAAATCTTATTGgtggaattgtg agcggata	<i>URA3</i> integration in Chr3R (between <i>AAP1</i> and <i>CTN3</i>) for LOH induction
Chr3RB-5DR	GGAAGAGGCGGAAAAATGACCAA AATGAAAATTTTTTTTTTACCTCAT TGATTTGCACAACATAGACAGACA GACACACCTACgtttccagtcacgacgtt	<i>URA3</i> integration in Chr3R (between <i>orf19.5866</i> and <i>WSC1</i>) for LOH induction
Chr3RB-3DR	GAATTAATGAATGAATAAATGAAT AAATGAATGAATAAGATAATTTGA TAAAAATCTTTCTGATTTTTTTTTTA AAAAGGAGAAGGAGgtggaattgtgagcg gata	<i>URA3</i> integration in Chr3R (between <i>orf19.5866</i> and <i>WSC1</i>) for LOH induction
19.3148-F	CAC TTCCTTCTTCTTCACACTTCA C	Checking of <i>URA3</i> integration in Chr2L
Chr3RA-F	GTTGAGATCTCAAACAAAACAAA AC	Checking of <i>URA3</i> integration between <i>AAP1</i> and <i>CTN3</i>
Chr3RB-F	GTAAGACCAGGTGGGACCAG	Checking of <i>URA3</i> integration between <i>orf19.5866</i> and <i>WSC1</i>
URA3det-R	GTATGGGGTTGTTGCTCAGG	Checking of <i>URA3</i> integration