A Stable Hybrid Containing Haploid Genomes of Two Obligate Diploid Candida Species

Uttara Chakraborty, Aiyaz Mohamed, Pallavi Kakade, Raja C. Mugasimangalam, Parag P. Sadhale, Kaustuv Sanyal

Molecular Mycology Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, India; Genotypic Technology (P) Ltd., Bangalore, India; Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India

Candida albicans and Candida dubliniensis are diploid, predominantly asexual human-pathogenic yeasts. In this study, we constructed tetraploid (4n) strains of C. albicans of the same or different lineages by spheroplast fusion. Induction of chromosome loss in the tetraploid C. albicans generated diploid or near-diploid progeny strains but did not produce any haploid progeny. We also constructed stable heterotetraploid somatic hybrid strains (2n + 2n) of C. albicans and C. dubliniensis by spheroplast fusion. Heterodiploid (n + n) progeny hybrids were obtained after inducing chromosome loss in a stable heterotetraploid hybrid. To identify a subset of hybrid heterodiploid progeny strains carrying at least one copy of all chromosomes of both species, unique centromere sequences of various chromosomes of each species were used as markers in PCR analysis. The reduction of chromosome content was confirmed by a comparative genome hybridization (CGH) assay. The hybrid strains were found to be stably propagated. Chromatin immunoprecipitation (ChIP) assays with antibodies against centromere-specific histones (C. albicans Cse4/C. dubliniensis Cse4) revealed that the centromere identity of chromosomes of each species is maintained in the hybrid genomes of the heterotetraploid and heterodiploid strains. Thus, our results suggest that the diploid genome content is not obligatory for the survival of either C. albicans or C. dubliniensis. In keeping with the recent discovery of the existence of haploid C. albicans strains, the heterodiploid strains of our study can be excellent tools for further species-specific genome elimination, yielding true haploid progeny of C. albicans or C. dubliniensis in future.

Generally harmless commensals sometimes become virulent in humans with compromised immune systems. Candida species belong to such a class of opportunistic yeast pathogens in humans (1). Candida albicans is one of the most frequently isolated fungal species from immunocompromised patients (2). Candida dubliniensis, another species that belongs to the same CTG clade (1), is most closely related to C. albicans (3) but less efficient in colonization and tissue invasion (4, 5). Among the many common features that these two species share, the presence of the same key mating genes (MTL loci) is particularly striking, considering their mostly asexual nature of propagation (6–8). Under laboratory conditions, it was shown that C. albicans has a parasexual cycle, which provides an alternative pathway to generate strain diversity (9). An elaborate mating system promotes conjugation between mating-competent opaque cells homozygous (a/a or α/α) for opposite mating types in this species (10). The resulting tetraploid strains undergo random yet concerted chromosome loss in order to return to the diploid or a near-diploid state. Genotype strains resulting from this parasexual cycle showed altered morphology on laboratory media at different temperatures, demonstrating that this mode of propagation can produce phenotypic variants (11). Such an alternative pathway to meiosis was thus a means to promote a reduction in the ploidy state in this organism. Intriguingly, meiosis was not observed either in C. albicans or in C. dubliniensis even when certain tetraploid strains of C. albicans undergoing a parasexual cycle exhibited Spo11-dependent genetic recombination between homologous chromosomes (11).

While C. albicans is the primary cause of a wide spectrum of mucocutaneous diseases in the immunosuppressed host body, C. dubliniensis has also been implicated under such conditions (7). Several experiments revealed that the MTLα and MTLa strains in each individual species (12) could be engineered to mate either in vitro or in vivo (13). However, despite establishing mating between C. albicans and C. dubliniensis, its occurrence in nature is yet unknown. Discovery of the MTLα/MTLα locus in C. dubliniensis with similarly arranged genes homologous to C. albicans genes eventually led to demonstration of interspecies mating between the two, both in suspension and on mouse skin (8). Prior to the discovery of mating in C. albicans, somatic hybridization demonstrated that tetraploids could be formed by means of spheroplast fusion (14, 15), and the products of chromosome loss (induced by artificial means) were also cells with a diploid or close to diploid DNA content, indicating that random segregation of chromosomes can occur in tetraploids generated either by mating or by fusion of spheroplast cells.

Since C. albicans and C. dubliniensis exhibit an amazing range of karyotypic rearrangements and can tolerate a substantial level of aneuploidy (16–18), an important aspect to study would be the mechanism of chromosome transmission during the parasexual mode of the cell cycle in these organisms. The process of chromosome segregation in mitosis and meiosis is largely powered by a dynamic kinetochore–microtubule interaction. The centromeres of C. albicans and C. dubliniensis chromosomes were identified to be the binding sites of their respective centromeric histone
sis state can be created from a hybrid of whether an altered ploidy state other than the diploid or tetraploid initated remains unknown. In our study, we sought to investigate

![](image)

**FIG 1** Somatic hybridization followed by parasexual chromosome loss as a means to generate intra- and interspecies progeny hybrid strains. (A) Haploid *C. albicans* (Ca) or *C. dubliniensis* (Cd) strains cannot be generated by means of meiosis; however, haploid *C. albicans* strains can exist in nature, but the mechanism for this is unknown. (B) Schematic to create heterodiploid (*n* + *n*) strains carrying the haploid genome content of each species. (C) Strategy for constructing an intra- or interlineage tetraploid somatic product of *C. albicans* or a heterotetraploid hybrid between *C. albicans* and *C. dubliniensis*. These hybrid products were further used for generation of progeny strains after induction of chromosome loss using a method described before (9) (see Materials and Methods).

![Diagram of somatic hybridization](image)

**A** Unknown

![](image)

**B** Ca (2n) + Cd (2n)

Spheroplast Fusion

CaCd somatic hybrid

(2n + 2n)

**C** Intra-species Hybridization

Intra-lineage

Ca

ura/+ URA/ura

Spheroplast Fusion

CaCd somatic hybrid

(2n + 2n)

Intr-species Hybridization

Ca

ura/+ URA/ura

Induction of chromosome loss

Progeny hybrids (n + n)

![Diagram of progeny hybrids](image)

**Materials and Methods**

A complete description of all materials and methods can be found in the supplemental material.

(Ch3H3) homologs, *C. albicans* Cse4 (CaCse4) and *C. dubliniensis* Cse4 (CdCse4) (2, 19). The properties of the centromeres in these organisms are different from those of the centromeres of most other species studied thus far, with each of the eight chromosomes carrying a unique CEN region rich in CenH3 molecules (20). Thus, each CEN can be used as a chromosome-specific marker to identify individual chromosomes of each of these two Candida species. Recently, it has been shown that even though the CEN regions are longer in *C. albicans* than *Saccharomyces cerevisiae*, only one microtubule is associated per kinetochore in both species (21). It is possible that the unique CEN properties along with the single kinetochore-microtubule interaction make the chromosome segregation machinery flexible, accommodating a wide range of variations in chromosome number of *C. albicans*.

A recent report suggests that *C. albicans* can rarely exist in the haploid state, which is unstable, and haploid isolates often switch to the diploid state (22). However, how these haploid strains originated remains unknown. In our study, we sought to investigate whether an altered ploidy state other than the diploid or tetraploid state can be created from a hybrid of *C. albicans* and *C. dubliniensis*. A haploid strain of either *C. albicans* or *C. dubliniensis* has not yet been generated by either a sexual or a parasexual process (Fig. 1A). In order to bypass this obstacle, a hybrid strain of these two diploid Candida species was created by spheroplast fusion (Fig. 1B). Subsequently, this somatic heterotetraploid hybrid can be induced to lose chromosomes through the process of parasexual reduction, which may generate a possible heterodiploid hybrid progeny strain with the haploid chromosome complement of each species. Here, we report on such a hybrid strain that carries either two sets (heterotetraploid) or one set (heterodiploid) of the chromosome complement of each of *C. albicans* and *C. dubliniensis* (Fig. 1C).

**Animals.** Female BALB/c mice (Mus musculus) approximately 6 to 8 weeks old were maintained and bred under pathogen-free conditions.

**Ethics statement.** Use of mice was approved by the Animal Ethics Committee of Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India. All animal experiments were performed according to the National Regulatory Guidelines issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

**Strains and media.** The strains of *C. albicans* and *C. dubliniensis* used in this study are listed in Table 1. J118 was constructed by deleting one copy of the DAD2 (orf19.3551) gene (present on CaChr2) by HIS1, and the other copy was tagged by the tandem affinity purification (TAP) tag at the C terminus with URA3 as the marker in strain BWP17. The resulting strain (J118) thus created is heterozygous for the URA3 gene (23). These strains were grown in 1% yeast extract–2% peptone–2% dextrose (YPD) with 10 mg/ml uridine supplement [YPD(U)] for routine growth or in supplemented synthetic dextrose (SD) minimal medium as described previously (19). Chromosome loss induction was performed with *S. cerevisiae* preporulation (prespo) medium, which contains 1% yeast extract, 0.8% peptone, and 10% dextrose, and 1-sorbose-containing medium (0.7% yeast nitrogen bases without amino acids, 2% 1-sorbose, 2% agar), as described previously (9). Screening for the diploid hybrid progeny was performed in SD minimal medium supplemented with uridine and 5-fluoroorotic acid (5-FOA). The media used to observe growth and hyphal induction in this study were CHROMagar, Spider medium, and YPD plus serum. All details about the composition of these media have been provided elsewhere (24).

**Construction of hybrid strains.** Spheroplasts were isolated and fused in *S. cerevisiae* according to a standard protoplast fusion protocol (25), along with a few modifications. Approximately 50 ml (containing about 5 × 10^6 cells) of exponential-phase cultures grown in YPD(U) was harvested, washed by centrifugation, and suspended in spheroplasting buffer in each case. This suspension was treated with 0.01% beta-mercaptoethanol and 20 μg/ml lyticase (Sigma Aldrich) and incubated at 30°C for 1 h. Spheroplasts of the two auxotrophic strains were mixed, harvested, treated with an equal volume of 30% polyethylene glycol (PEG; molecular weight, 3,350), and incubated at room temperature for 10, 20, or 30 min. Following PEG treatment, several washes were given to the mixed cell pellet in MP buffer (1 M sorbitol, 0.1 M NaCl, 0.01 M acetic acid). The
TABLE 1 Strains used in this study*

| Strain     | Genotype                                                                 | Reference |
|------------|--------------------------------------------------------------------------|-----------|
| SC5314     | Wild type                                                               | 37        |
| J118       | Δura3::imm434/Δura3::imm434 Δhis1::hisGΔhis1::hisG Δarg4::hisGΔarg4::hisGDAD2-TAP URA3/ORF19.551::HIS1 | 23        |
| Wu284      | Clinical isolate                                                        | 38        |
| GUUM4a     | URA3::FRT/ura3::FRT                                                     | 39        |
| BWPL1      | Δura3::imm434/Δura3::imm434 Δhis1::hisGΔhis1::hisG Δarg4::hisGΔarg4::hisG | 40        |
| WUM1B      | URA3-1::ura3-2 Δ::MPA-FLIP                                             | 41        |
| UWM5A      | ura3-1::FRT/ura3-2 Δ::FRT                                              | 41        |
| RM1000     | CAI4 ura3::λ imm434/ura3::λ imm434 hist1::hisG/hist1::hisG              | 42        |
| URM1000    | RM1000 RPS10/rps10::URA3                                               | This study|
| HBT1       | Prototroph, tetraploid somatic hybrid of J118 and GUUM4b               | This study|
| HBT2       | Prototroph, tetraploid somatic hybrid of J118 and GUUM4b               | This study|
| CAT1       | Prototroph, tetraploid somatic hybrid of J118 and RM1000               | This study|
| CAT2       | Prototroph, tetraploid somatic hybrid of J118 and RM1000               | This study|
| CAT3       | Prototroph, tetraploid somatic hybrid of BWPL1 and WUM1B               | This study|
| CAP101 to CAP204 | ura3, progeny of CAT1 and CAT2                                        | This study|
| UCAP101    | CAP101 RPS10/rps10::URA3                                              | This study|
| UCAP102    | CAP102 RPS10/rps10::URA3                                              | This study|
| UCAP103    | CAP103 RPS10/rps10::URA3                                              | This study|
| UCAP104    | CAP104 RPS10/rps10::URA3                                              | This study|
| UCAP201    | CAP201 RPS10/rps10::URA3                                              | This study|
| UCAP202    | CAP202 RPS10/rps10::URA3                                              | This study|
| UCAP203    | CAP203 RPS10/rps10::URA3                                              | This study|
| UCAP204    | CAP204 RPS10/rps10::URA3                                              | This study|
| CAP301 to CAP321 | ura3 progeny of CAT3                                                  | This study|
| UCAP301    | CAP301 RPS10/rps10::URA3                                              | This study|
| UCAP302    | CAP302 RPS10/rps10::URA3                                              | This study|
| UCAP303    | CAP303 RPS10/rps10::URA3                                              | This study|
| UCAP304    | CAP304 RPS10/rps10::URA3                                              | This study|
| UCAP305    | CAP305 RPS10/rps10::URA3                                              | This study|
| UCAP306    | CAP306 RPS10/rps10::URA3                                              | This study|
| UCAP307    | CAP307 RPS10/rps10::URA3                                              | This study|
| UCAP308    | CAP308 RPS10/rps10::URA3                                              | This study|
| UCAP309    | CAP309 RPS10/rps10::URA3                                              | This study|
| UCAP310    | CAP310 RPS10/rps10::URA3                                              | This study|
| HBP1 to HBP30 | ura3 progeny HBT1, derived from prespo and 1-sorbose media              | This study|
| UHBP1      | HBP1 RPS10/rps10::URA3                                                | This study|
| UHBP2      | HBP2 RPS10/rps10::URA3                                                | This study|
| UHBP3      | HBP3 RPS10/rps10::URA3                                                | This study|
| UHBP4      | HBP4 RPS10/rps10::URA3                                                | This study|
| UHBP5      | HBP5 RPS10/rps10::URA3                                                | This study|
| UHBP6      | HBP6 RPS10/rps10::URA3                                                | This study|
| UHBP7      | HBP7 RPS10/rps10::URA3                                                | This study|
| UHBP8      | HBP8 RPS10/rps10::URA3                                                | This study|
| UHBP9      | HBP9 RPS10/rps10::URA3                                                | This study|
| UHBP10     | HBP10 RPS10/rps10::URA3                                               | This study|
| UHBP11     | HBP11 RPS10/rps10::URA3                                               | This study|

* FRT, flippase recombination target; MPA, mycophenolic acid; MPAr-FLIP, MPA resistant flipper. Ura" derivatives have U as a prefix to their names.

Fused spheroplast suspension was subsequently mixed in the molten regeneration medium (0.67% yeast nitrogen base, 2% dextrose, 18.2% sorbitol, 1.2% agar) and finally overlaid on selective synthetic medium (complete medium [CM], uridine, arginine).

Induced chromosome loss experiment. To test for chromosome loss, the tetraploid hybrid strains obtained by somatic fusion were grown on different media, such as S. cerevisiae prespo medium and 1-sorbose medium, and incubated at 30°C and 37°C for 7 to 15 days (9). Following incubation, cells were streaked on synthetic medium containing uridine and 5-FOA. Chromosome loss was observed in three independent experiments with cells grown on prespo or 1-sorbose medium.

Flow cytometry, cytological analysis, and indirect immunofluorescence. Asynchronous cultures of the strains were grown in YPD(U) to an A600 of 0.6 and processed for flow cytometry and other cytological analysis as described before (2). Intracellular Cse4 was visualized by indirect immunofluorescence as described previously (2) (details of the procedure are given in the supplemental material).

ChIP assay. Chromatin immunoprecipitation (ChIP) followed by PCR analysis was performed as described previously (19, 20).

Virulence assay in mouse model for systemic candidiasis. Cultures of strains used in the virulence assay were grown in YPD(U) to an A600 of 1.000.
Preparation of inoculum for injection. BALB/c mice (females; age, 6 to 8 weeks; weight, approximately 20 to 22 g) were injected through the lateral tail vein (26). Approximately 10 ml cultures were centrifuged at 4,000 rpm for 5 min to pellet down the cells at room temperature. The cell pellets were resuspended in 10 ml 0.85% saline, and the actual concentration of cells was verified by counting the cells in a hemocytometer and by plating to determine the viable cell count. Different concentrations of cells (5 × 10^6 cells/50 μl, 5 × 10^7 cells/50 μl) were intravenously injected into the tail vein of each mouse. Each Candida strain was injected into four or five mice at each of the concentrations of cells indicated above. Survival was monitored after every 6 to 8 h. Readings were taken on the basis of at least four separate experiments with each strain tested. The survival curves were compared among the different strains by using the log-rank (Mantel-Cox) test (Prism software, version 5.0). P values calculated by this test along with the survival curves are indicated in Fig. 7.

Microarray data accession number. A custom comparative genome hybridization (CGH) microarray, the C. albicans and C. dubliniensis Cross-Species CGH microarray (4 × 180K), was designed using an Agilent platform (see the supplemental material). Microarray data have been submitted to the GEO database (http://www.ncbi.nlm.nih.gov/geo/) with accession number GSE24269.

RESULTS

An induced parasexual cycle in C. albicans and C. dubliniensis resulted in the generation of interspecific somatic hybrid progeny. We performed PEG-mediated somatic hybridization between diploid C. albicans strains belonging to the same or different lineages (Fig. 2A and B; Table 2). Flow cytometry indicated that two strains, CAT1 and CAT2 (where CAT represents C. albicans tetraploid), obtained from intralineage fusion and CAT3, obtained from interlineage fusion, were tetrapioids (Fig. 2, right; Table 1 and Table 2). Two culture conditions (9) were used to induce chromosome loss in them: Saccharomyces cerevisiae prespo and ϕ-sorbose-containing synthetic media (at 30°C and 37°C). All the tetrapioid strains had only one functional copy of the URA3 gene on C. albicans chromosome 2 (CaChr2). To select for progeny that might have undergone parasexual chromosome loss, colonies from prespo and ϕ-sorbose media were incubated in 5-FOA-containing medium supplemented with uridine. Loss of at least this chromosome should allow growth on the 5-FOA-containing medium. Four progeny strains were obtained from each of CAT1 grown on prespo medium (CAP101 to CAP104, where CAP represent C. albicans progeny) and CAT2 grown on ϕ-sorbose medium (CAP201 to CAP204) (Table 2). Twenty-one progeny strains (CAP301 to CAP321) were derived from CAT3 (Table 2) after induced chromosome loss; of these, 18 were from prespo medium and the others were from ϕ-sorbose medium. However, progeny strains isolated from either medium were phenotypically indistinguishable. Flow cytometric measurement of the DNA content of these C. albicans progeny strains (Table 2) indicated that the ploidy of these strains belonged to two classes: (i) 2n to 4n and (ii) ~2n (Fig. 3A; see Fig. S1A and B in the supplemental material).

Using a similar strategy, we generated interspecies hybrid strains of C. albicans and C. dubliniensis. Spheroplasts obtained from C. albicans strain J118 (URA/ura arg/arg), a derivative of BW17, and C. dubliniensis strain CdUM4b (ura/ura ARG/ARG), a derivative of Wu284, were fused (Fig. 2C). The fusant cells first appeared to form syncytia, a large undivided, multinucleated mass that the ploidy of these strains belonged to two classes: (i) 2n to 4n and (ii) ~2n (Fig. 3A; see Fig. S1A and B in the supplemental material).

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FIG 2 Flow cytometry (fluorescence-activated cell sorting) analyses determined the ploidy of the various somatic hybrid strains. (A) Schematic representing intralineage spheroplast fusion between J118 and RM1000 generating homotetraploid fusion products CAT1 and CAT2. (B) Schematic representing interlineage spheroplast fusion between UBWP17 and WUM5A generating homotetraploid fusion product CAT3. (C) Schematic representing interspecies spheroplast fusion between J118 and CdUM4b generating heterotetraploid fusion products HBT1 and HBT2. The ploidy of all the parents and their respective hybrid strains was determined by fluorescence-activated cell sorter analyses (right). For ploidy analysis, cells were stained with PI to examine the cellular DNA content. The x axis of each graph (PI) represents a linear scale of fluorescence, and the y axis (counts) represents a linear scale of cell number.
TABLE 2 Description of strains constructed in this studya

| Nature of fusion | Parent 1 | Parent 2 | Product | Progeny | Uraa progenyb |
|------------------|----------|----------|---------|---------|---------------|
| Intralineage     | J118 (C. albicans) | RM1000 (C. albicans) | CAT (4n) | CAP101 to CAP104 | UCAP101 to UCAP104 |
| Intralineage     | J118 (C. albicans) | RM1000 (C. albicans) | CATZ (4n) | CAP201 to CAP204 | UCAP201 to UCAP204 |
| Interlineage     | UBPW17 (C. albicans) | WUMSA (C. albicans) | CAT3 (4n) | CAP501 to CAP521 | UCAP501 to UCAP530 |
| Interspecies     | J118 (C. albicans) | CdUM4b (C. dubliniensis) | HBT1 (2n + 2n) HBT2 (2n + 2n) | HBH1 to HBP30 | UHBP1 to UHBP11 |

a Uraa derivatives have U as a prefix to their names.

b Not determined.

doed cells (see Fig. S2A in the supplemental material), completed nuclear fusion, and were subsequently found to be mononuclear (see Fig. S2A to D in the supplemental material). Analysis of propidium iodide (PI)-stained cells by flow cytometry indicated that two hybrid strains, HBT1 and HBT2 (where HBT represents hybrid tetraploid; Table 2), were heterotetraploid (2n + 2n) (Fig. 2, right; see Fig. S3A in the supplemental material). The stability of these homo- and heterotetraploid hybrid strains was measured by passaging them in nonselective media for at least 30 generations, and the ploidy of the cells was examined after each passaging by flow cytometry (see Fig. S4 in the supplemental material). No observable change in the ploidy states of these strains was seen after passaging. By inducing chromosome loss, we further constructed hybrid progeny strains from HBT1 with reduced DNA content. Thirty progeny strains were obtained from HBT1 (HBP) to HBP30, where HBP represents hybrid progeny, with 24 strains being derived from prespo medium and 6 strains being derived from 1-sorbose medium (Table 2). They belonged to two categories of ploidy content: (i) (n + n) to (2n + 2n) and (ii) close to (n + n) (Fig. 3B; see Fig. S1C in the supplemental material). We studied nuclear division in DAPI (4',6-diamidino-2-phenylindole)-stained cells of these strains (Table 3) and found no visible nuclear segregation defects in any case.

When grown at 30°C in rich medium, colonies from different progeny strains displayed a wide range of phenotypes, from smooth (more of the yeast-phase cells) to wrinkled (more of the filamentous cells) colonies (Fig. 4A). Microscopic studies revealed the presence of both yeast and pseudohyphal cells in wrinkled colonies, while the smooth colonies had a majority of the yeast cells (see Fig. S5 and S6 in the supplemental material). In order to clinically demarcate the heterodiploid progeny strains from their wild-type and hybrid parents, we used CHROMagar, a commercially available chromogenic agar-based medium, and grew the strains at 37°C. C. albicans colonies were Persian green, and C. dubliniensis colonies appeared dark blue. Colonies of C. dubliniensis were significantly more slowly growing at 37°C than those of C. albicans (data not shown). HBH1 developed an intermediate coloration and formed a hyphal mat in medium (Fig. 4B). The heterodiploid progeny could easily be differentiated from their homodiploid counterparts.

PCR analysis with chromosome-specific CEN primer pairs (see Table S1 in the supplemental material) confirmed that HBH1 and HBH2 had each of the 8 chromosomes of C. albicans and C. dubliniensis (Fig. 3C; see Fig. S3 in the supplemental material). Of all the progeny hybrid strains derived from HBH1, 22 heterodiploid progeny strains with close diploid DNA content retained all 16 CEN regions from both parents, indicating that at least one copy of each of the C. albicans and C. dubliniensis chromosomes was present. Similar analysis revealed that at least 6 progeny strains had lost both homologs of certain chromosomes of either C. albicans or C. dubliniensis (see Fig. S7 in the supplemental material). Since these strains were viable, cross-species functional complementation of lost chromosomes must have occurred. We tested the doubling time of each of these strains of various classes obtained through this process (see Table S2 in the supplemental material). An increase in the generation time of the tetraploid and heterotetraploid strains and their progeny strains (105 to 125 min) compared to that of C. albicans (95 min) or C. dubliniensis (90 min) was observed. It should be noted that this increase in doubling time observed in the hybrid progeny compared to that observed in their diploid parent strains was not due to the absence of the URA3 gene.
TABLE 3 Percentage of cells with the indicated nuclear morphologies based on DAPI staining

| Strain       | %Unbudded | %Small budded | %Properly segregated nuclei | %Improperly segregated nuclei | Total no. of cells |
|--------------|-----------|---------------|-----------------------------|-------------------------------|-------------------|
| J118         | 68        | 14            | 17.6                        | 0.4                           | 260               |
| GUM14b       | 21        | 45            | 34                          | 13                            | 139               |
| RM10000      | 70        | 16            | 13                          | 1                             | 100               |
| UBWP17       | 75        | 10            | 15                          | 120                           | 120               |
| WUM5A        | 22.5      | 52.5          | 25                          | 200                           | 200               |
| HBT1         | 55        | 24            | 18                          | 3                             | 293               |
| UHB1P        | 13        | 33            | 50                          | 4                             | 278               |
| CAT1         | 45        | 14            | 36                          | 5                             | 188               |
| UCAP102      | 53        | 28            | 13.4                        | 5.6                           | 142               |
| CAT3         | 34        | 48            | 13                          | 5                             | 258               |
| UCAP301      | 75        | 15            | 8                           | 2                             | 300               |

Parasexual chromosome loss resulted in a euploid or aneuploid copy number of chromosomes. To determine the copy number of each chromosome of *C. albicans* and *C. dubliniensis* in HBT1 (prototroph) and HBP1 (a Ura− heterodiploid progeny), we performed array comparative genome hybridization (aCGH) using custom-designed whole-genome microarrays representing the *C. albicans* and *C. dubliniensis* genomes (see Materials and Methods). It was evident that the copy number of all chromosomes in HBT1, based on *C. albicans*-specific probes (see Table S3 in the supplemental material), was close to 2 (range, 1.6 to 2.2) (Fig. 5A). We adapted *C. dubliniensis* probes based on the chromosome annotation that was submitted to EMBL (http://www.ebi.ac.uk/embl/) (3), and most of the chromosomes showed copy numbers close to 2 (range, 1.5 to 2.0), except for *C. dubliniensis* chromosome 4 (CdChr4) and CdChr6 (Fig. 5B; see Table S3 in the supplemental material). The *C. dubliniensis* annotated chromosome assembly (based on the sequenced strain, Cd36) was used here for the *C. dubliniensis* probes while designing the tiling arrays relative to each other. The relative copy number of most of the *C. albicans* and *C. dubliniensis* chromosomes (12 out of 16 chromosomes) in HBP1 was found to be close to 1 (Fig. 5C and D); the exceptions were for CaChr2, CdChr2, CdChr3, and CdChr4, where the values were found to be less than 1 (see Table S3 in the supplemental material). It is possible that these numbers do not actually reflect the absolute number of copies of specific chromosomes per cell; rather, they may indicate an overall greater variation in chromosomal composition in the population of cells of HBP1. This heterodiploid progeny was found to have half the genome content of the heterotetraploid (for *C. albicans* chromosomes, 0.56 ± 0.10; for *C. dubliniensis* chromosomes, 0.45 ± 0.13) (see Table S3 in the supplemental material). It is to be noted here that the *C. dubliniensis* parent strain used in this study is a derivative of WU284, and as two different *C. dubliniensis* clinical isolates differ significantly in karyotype (27), the copy number differences observed in certain *C. dubliniensis* chromosomes of the hybrid genome can well be expected.

The chromosomes of the hybrid strains segregate faithfully during mitosis. Since high-fidelity chromosome segregation depends on proper centromere formation on a chromosome, we examined binding of the centromeric histones (CenH3) CaCse4 and CdCse4 at the centromeres of all the chromosomes from *C. albicans* and *C. dubliniensis* in the hybrid and its progeny strains. Indirect immunofluorescence microscopy using affinity-purified polyclonal anti-CaCse4 or anti-CdCse4 antibodies (against amino acid residues 1 to 18 of CaCse4) (2) revealed bright dot-like signals in all the cells (Fig. 6) of the interspecies hybrid as well as its representative progeny. The localization patterns appeared to be identical to those of CaCse4 in *C. albicans* (2) and CdCse4 in *C. dubliniensis* (19) at corresponding stages of the cell cycle (Fig. 6A and B). Coimmunostaining of fixed cells with antitubulin and anti-Ca/CdCse4 antibodies showed proper spindle morphology in the dividing cells, analogous to the typical localization patterns of kinetochore proteins in *C. albicans* and *C. dubliniensis*. Standard ChIP assays with anti-Ca/CdCse4 antibodies revealed enrichment of Cse4 on the CEN regions of *C. albicans* and *C. dubliniensis*. Standard ChIP-PCR analysis revealed significant enrichment of CaCse4 and CdCse4 to their native CEN loci on all the respective chromosomes of the hybrid except in chromosome 4 of *C. dubliniensis* (Fig. 6C to F). Binding of the evolutionarily conserved kinetochore protein CaCse4 or CdCse4 to each of the species-specific CEN regions strongly suggests that the centromere identity of the chromosomes of both the species is maintained in these hybrid strains.

Homotetraploid *C. albicans* strains show better fitness as pathogens than their heterotetraploid counterparts. Virulence is a measurable trait of the biological fitness of pathogenic organisms. In this study, we sought to compare the biological fitness of these yeasts in their natural ploidy states with that in an altered one. Of all the biological processes thought to be related to virulence, the ability to show filamentous growth from yeast cells both *in vivo* and *in vitro* is considered to be an important trait in *C. albicans* and *C. dubliniensis* (5). Both grow as budding yeasts in standard growth medium, YPD, at 30°C. In response to various stimuli, for instance, elevated temperatures, the presence of serum, or nutrient starvation conditions, these yeasts switch to filamentous forms.

In this study, we compared the growth patterns of the hybrid strains with those of their diploid parent strains under 3 different temperature conditions: 30°C, 37°C, and 42°C (Fig. 4A). *C. albicans* tetraploids and their progeny strains showed proper growth at all the temperatures, with CAT1 and its UCAP102 progeny strain (Ura− derivatives have U as a prefix to their names) being significantly hyphal at 42°C (see Fig. S5 and S6 in the supplemental material). On the other hand, CdUM4b did not grow at this temperature at all, as expected from previous reports of *C. dubliniensis* (7). In contrast to their parents, cells of the heterotetraploid hybrid and its UHB1 progeny were constitutively filamentous when grown at all these temperatures (see Fig. S6 in the supplemental material). Extensive filamentation in these two strains caused flocculation in broth at both 37°C and 42°C (Fig. 4A; see Fig. S6 in the supplemental material). These two strains essentially grew as budding yeast cells at a lower temperature, such as 28°C.

When they were compared for their colony morphologies in different morphology media (24), YPD plus serum at 37°C in-
duced wrinkled colony formation in the diploid C. albicans strains heterozygous for URA3, suggesting a mix of yeast and hyphal cells (Fig. 4A). All the ura3 mutants grew as smooth colonies under similar conditions of growth. However, the tetraploid derivatives and their corresponding Ura+ C. albicans progeny also grew as smooth colonies, suggesting the presence of a majority of yeast cells. In contrast to these strains, the heterotetraploid and its progeny strains always showed a wrinkled hyphal morphology in all these media. Here again we observed that they were mostly filamentous under the above-described conditions (Fig. 4A; see Fig. S5 and S6 in the supplemental material).

In keeping with these observations, we were interested to see if this filamentous growth phenotype affected the virulence of the strains in any way or not. As the uridine auxotrophs of C. albicans strains showed compromised pathogenicity, C. albicans URA3 was integrated into the CaChr1 RPS10 locus (28) of all the parent Ura− strains (see the Materials and Methods in the supplemental material). All animal experiments were performed with Ura+ strains of both C. albicans and C. dubliniensis. A tetraploid C. albicans strain has previously been shown to be either as virulent as or less virulent than the diploid parent strains in a mouse model of systemic infection (29). We compared the virulence potential of the strains having altered ploidy with that of their diploid Candida parent strains. An intravenous dosage of 5 × 10⁵ cells of SC5314 (C. albicans) was sufficient to kill mice within 12 days postinfection, whereas the same dosage of Wu284 (C. dubliniensis) cells could not (Fig. 7A). However, at a higher dosage of 5 million cells, both strains killed the animals within 3 to 4 days postinfection (Fig. 7B).

Thus, a dosage of 5 million cells of each strain was administered intravenously in a murine systemic model of infection. The viru-
The virulence potential of the intra- and interlineage tetraploids (CAT1, CAT2, and CAT3) was comparable to that of their wild types at this dosage (Fig. 7; see Table S4 in the supplemental material). Their progeny strains also showed a 100% mortality rate by a maximum of 2 weeks. Thus, the difference in ploidy did not seem to have any significant effect on the virulence potential of C. albicans. The virulence of interspecies tetraploid strain HBT1 (C. albicans and C. dubliniensis) was also similar to that of the intraspecies counterparts at this dosage in the animals. However, differences between the two were observed at a lower dosage. When $5 \times 10^5$ cells of HBT1 were injected into mice, they did not kill the animals, whereas the same dosage of C. albicans tetraploids did. This indicated that the virulence of the heterotetraploid hybrid carrying two sets of C. albicans chromosomes was less than that of the homotetraploid C. albicans strain (Fig. 7C; see Table S4 in the supplemental material). The virulence levels of the URA3 integrated homodiploid and heterodiploid progeny strains were also compared (see Table S4 in the supplemental material). While the C. albicans hybrid progeny showed different levels of virulence in mice, the heterodiploid progeny set of strains from this lineage had, remarkably, become avirulent.

**DISCUSSION**

C. albicans and C. dubliniensis are mostly obligate diploid yeasts. A haploid derivative of C. albicans or C. dubliniensis could not be generated by a sexual or parasexual process. In order to achieve this, we first adapted a method to generate both intra- and interlineage C. albicans tetraploid strains by somatic hybridization. In-
duction of chromosome loss generated stable diploid progeny of C. albicans strains. We applied this strategy to develop a heterotetraploid strain, a hybrid of diploid C. albicans and C. dubliniensis. Surprisingly, induction of chromosome loss in this strain generated a major class of stable progeny strains that were heterodiploid, presumably carrying a haploid set of chromosomes of each species. aCGH analysis confirmed the haploidization of the chromosome content in the heterodiploid species. The hybrid strains maintained the centromere identity and segregated chromosomes stably through many generations. We demonstrated in this study that a C. albicans or C. dubliniensis strain can exist with only a haploid genome content of each species, as in a hybrid.

Having created such stable strains that differ in chromosome content and the ploidy state, we further examined the biological fitness of these strains using virulence as a measurable attribute. Using a murine model of systemic infection, we concluded that a strain carrying a diploid set of chromosomes of C. albicans or C. dubliniensis is almost always virulent either as an individual species or as a hybrid (the heterotetraploid strain), whereas heterodiploid progeny hybrids from this lineage carrying a haploid set of chromosomes of each species are avirulent. The loss of virulence in heterodiploid strains observed in this study could be due to loss of a functional allele of a virulence gene of each species. An alternative explanation could be a requirement for a diploid genome.
content to maintain the dosage of genes of each species required for virulence. Subsequently, we examined other measurable fitness parameters in these strains, such as morphogenetic switching and growth under various conditions. While the heterotetraploid strains and most heterodiploid progeny strains showed no defects in switching, some heterodiploid strains showed a C. dubliniensis-like nonswitching phenotype. At 42°C, C. dubliniensis strains did not grow, but all the hybrid strains could proliferate without any significant delay.

This report describes several striking observations. First, there was a high propensity among the heterodiploid progeny strains to lose an entire haploid set of chromosomes from a species. Centromeres are clustered in C. albicans (2, 23, 30, 31) and C. dubliniensis (19). The 5-FOA selection performed in this study probably enriches a population of cells where centromere clustering is somehow compromised, leading to the loss of the URA3-containing chromosome. It has previously been shown that S. cerevisiae chromosomes physically interact with each other through similarly clustered centromeres (32). Assuming that an analogous interchromosomal interaction through clustered centromeres exists in Candida, loss of URA3-containing chromosome 2 might have made the cell prone to lose the chromosomes attached to it. Thus, although the mechanism of such a highly biased pattern of chromosome loss is uncertain, this finding suggests that loss of a single chromosome perhaps predisposes the genome to lose a complete haploid set of chromosomes. Hence, parasyexual chromosome loss is more of a concerted rather than a random phenomenon (11). Based on the PCR-based analysis using the CEN-specific primers, we also found that some progeny strains lack both homologs of one of the two species. The frequency of loss of both homologous chromosomes of one species is also biased. These results suggest that cross-species complementation of an entire chromosome is possible for some chromosomes of each species.

Second, the heterodiploid hybrid progeny were as stable as the diploid C. albicans or C. dubliniensis strains, in terms of growth potential or viability, as the dosage of most genes was probably compensated for by the homologous genes of the other species. However, there was variation in ploidy between the intra- and interlineage homodiploid progeny population. This was probably due to the genome instability of the parent strains, which has already been documented (33). In terms of their virulence properties, 1 out of 10 progeny strains of CAT3 tested on mice did not kill the animals but the animals showed signs of chronic infection, while another strain took a longer time than the intralineage ones to show 100% virulence. On the contrary, the Ura1 interspecies progeny strains remained avirulent even after URA3 integration. Since all these strains grew well at 37°C, the reason for the reduced virulence of the two progeny strains described above (CAT3 derivatives) could be due to homozgyosis of a mutant virulence-causing gene.

Third, the centromere identity of the chromosomes of each species is maintained in these hybrid strains. The hybrid condition did not reposition the centromeres, even though neo-centromeres have a high propensity to be formed in C. albicans chromosomes (34). ChiP analysis revealed that the centromere location of chromosome 4 changed between two C. dubliniensis strains, Cd36 (19) and GdUM4b. It should be noted here that this type of centromere repositioning has been demonstrated previously (35) and may have implications in the evolution of the centromere location during speciation. Identification of the altered centromere location in chromosome 4 of C. dubliniensis strain GdUM4b can provide us with new insight into centromere repositioning in yeasts.

Finally, to our knowledge, this is the first demonstration of the centromere identity of a species being maintained even in an interspecies hybrid genomic context, suggesting that the chromosome-specific centromere formation of each species can be maintained even in the presence of two different Cse4 proteins in the hybrid strains.

A recent study demonstrated that C. albicans can exist in the haploid state. Even though the haploid state seems to be unstable, under a controlled condition, the haploid C. albicans strain can propagate and even mate (22). However, in the absence of meiosis, the process that triggers haploidization of this largely obligate diploid organism is still an enigma. We can expand the prospects of this discovery and explore if a haploid strain of either species can be generated in a strategized manner or not. It has been shown that haploid fertile Arabidopsis thaliana plants can be developed by manipulating the centromere-specific histone, CENH3 (36). A similar strategy of deleting CenH3 (Cse4) of one species in the heterodiploid hybrid Candida strain that we created in this study may result in uniparental chromosome loss to generate a pure haploid C. albicans or C. dubliniensis strain in future.

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REFERENCES
1. Bennett RJ. 2010. Coming of age—sexual reproduction in Candida species. PLoS Pathog. 6:e1000155. doi:10.1371/journal.ppat.1000155.
2. Sanval K, Carbon J. 2002. The CENP-A homolog CaCse4p in the pathogenic yeast Candida albicans is a centromere protein essential for chromosome transmission. Proc. Natl. Acad. Sci. U. S. A. 99:12969–12974.
3. Jackson AP, Gamble JA, Yeomans T, Moran GP, Saunders D, Harris D, Aslett M, Barrell JF, Butler G, Citiulo F, Coleman DC, de Groot PWJ, Goonan MJ, Quail MA, McQuillan J, Munro CA, Pain A, Poulter RT, Rajandream M-A, Renaud H, Spiering MJ, Tivey A, Gow NAR, Barrell B, Sullivan DJ, Berriman M. 2009. Comparative genomics of the fungal pathogens Candida dubliniensis and Candida albicans. Genome Res. 19:2231–2244.
4. Moran GP, Stokes C, Thewes S, Hube B, Coleman DC, Sullivan D. 2004. Comparative genomics using Candida albicans DNA microarrays reveals absence and divergence of virulence-associated genes in Candida dubliniensis. Microbiology 150:3363–3382.
5. O’Connor L, Caplice N, Coleman DC, Sullivan DJ, Moran GP. 2010. Differential filamentation of Candida albicans and Candida dubliniensis is governed by nutrient regulation of UME6 expression. Eukaryot. Cell 9:1383–1397.
6. Sullivan DJ, Westereng TJ, Haynes KA, Bennett DE, Coleman DC. 1995. Candida dubliniensis sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. Microbiology 141:1507–1521.
7. Sullivan DJ, Moran GP, Pinjon E, Al-Mosaid A, Stokes C, Vaughan C, Coleman DC. 2004. Comparison of the epidemiology, drug resistance mechanisms, and virulence of Candida dubliniensis and Candida albicans. FEMS Yeast Res. 4:369–376.
8. Pujol G, Daniels KJ, Lockhart SR, Srikantha T, Radke JB, Geiger J, Soll DR. 2004. The closely related species Candida albicans and Candida dubliniensis can mate. Eukaryot. Cell 3:1015–1027.
9. Bennett RJ, Johnson AD. 2003. Completion of a parasexual cycle in Candida albicans by induced chromosome loss in tetraploid strains. EMBO J. 22:2505–2515.
10. Alby K, Schaef er D, Bennett RJ. 2009. Homothallic and heterothallic mating in the opportunistic pathogen Candida albicans. Nature 460:890–893.

11. Forche A, Alby K, Schaef er D, Johnson AD, Berman J, Bennett RJ. 2008. The parasysexual cycle in Candida albicans provides an alternative pathway to meiosis for the formation of recombinant strains. PLoS Biol. 6:e110. doi:10.1371/journal.pbio.0060110.

12. Hull CM, Johnson AD. 1999. Identification of a mating type-like locus in the asexual pathogenic yeast Candida albicans. Science 285:1271–1275.

13. Magee BB, Magee PT. 2000. Induction of mating in Candida albicans by construction of MTLa and MTL alpha strains. Science 289:310–313.

14. Sarachek A, Rhoads DS, Schwarzhoff R. 1981. Hybridization of Candida albicans through fusion of protoplasts. Arch. Microbiol. 129:1–8.

15. Sarachek A, Rhoads DS. 1981. Production of heterokaryons of Candida albicans by protoplast fusions: effects of differences in proportions and regenerative abilities of fusion partners. Curr. Genet. 4:221–222.

16. Chibana H, Beckerman JL, Magee PT. 2000. Fine-resolution physical mapping of genomic diversity in Candida albicans. Genome Res. 10:1865–1877.

17. Magee BB, Sanchez MD, Saunders D, Harris D, Berriman M, Magee P. 2008. Extensive chromosome rearrangements distinguish the karyotype of the hypovirulent species Candida dubliniensis from the virulent Candida albicans. Fungal Genet. Biol. 45:338–350.

18. Barton RC, Gull K. 1992. Isolation, characterization, and genetic analysis of monosomic, aneuploid mutants of Candida albicans. Mol. Microbiol. 6:171–177.

19. Padmanabhan S, Thakur J, Siddharthan R, Sanyal K. 2008. Rapid evolution of Cse4p-rich centromeric DNA sequences in closely related pathogenic yeasts, Candida albicans and Candida dubliniensis. Proc. Natl. Acad. Sci. U. S. A. 105:19797–19802.

20. Sanyal K, Baum M, Carbon J. 2004. Centromeric DNA sequences in the pathogenic yeast Candida albicans are all different and unique. Proc. Natl. Acad. Sci. U. S. A. 101:11374–11379.

21. Joglekar AP, Bouck D, Finley K, Liu X, Wan Y, Berman J, He X, Salmon ED, Bloom K. 2008. Molecular architecture of the kinetochore-centromere. Eukaryot. Cell 7:587–594.

22. Hickman MA, Zeng G, Forche A, Hirakawa MP, Abbey D, Harrison BD, Wang Y-M, Su C-H, Bennett RJ, Wang Y, Berman J. 2013. The ‘obligate diploid’ Candida albicans forms mating-competent haploids. Nature 494:55–59.

23. Thakur J, Sanyal K. 2011. The essentiality of the fungus-specific Dcm1 complex is correlated with a one-kinetochore-one-microtubule interaction present throughout the entire cell cycle, independent of the nature of a centromere. Eukaryot. Cell 10:1295–1305.

24. Homann OR, Dea J, Noble SM, Johnson AD. 2009. A phenotypic profile of the Candida albicans regulatory network. PLoS Genet. 5:e1000783. doi:10.1371/journal.pgen.1000783.

25. Curran BP, Bugjea VC. 1996. Protoplast fusion in Saccharomyces cerevisiae. Methods Mol. Biol. 53:45–49.

26. Csank C, Schroppel K, Leberer E, Harcus D, Mohamed O, Meloche S, Thomas DY, Whiteway M. 1998. Roles of the Candida albicans mitogen-activated protein kinase homolog, Cek1p, in hyphal development and systemic candidiasis. Infect. Immun. 66:2713–2721.

27. Gee SF, Joly S, Soll DR, Meis JFGM, Verweij PE, Polacheck I, Sullivan DJ, Coleman DC. 2002. Identification of four distinct genotypes of Candida dubliniensis and detection of microevolution in vitro and in vivo. J. Clin. Microbiol. 40:556–574.

28. Brand A, MacCallum DM, Brown AJP, Gow NAR, Odds FC. 2004. Ectopic expression of URA3 can influence the virulence phenotypes and proteome of Candida albicans but can be overcome by targeted reintegra-
tion of URA3 at the RPS10 locus. Eukaryot. Cell 3:900–909.

29. Ibrahim AS, Magee BB, Sheppard DC, Yang M, Kauffman S, Becker J, Edwards JE, Jr, Magee PT. 2005. Effects of ploidy and mating type on virulence of Candida albicans. Infect. Immun. 73:7366–7374.

30. Thakur J, Sanyal K. 2012. A coordinated interdependent protein circuitry stabilizes the kinetochore ensemble to protect CENP-A in the human pathogenic yeast Candida albicans. PLoS Genet. 8:e1002661. doi:10.1371/journal.pgen.1002661.

31. Roy B, Burrack LS, Lone MA, Berman J, Sanyal K. 2011. Cmt1p1, a member of the evolutionarily conserved Mis12 kinetochore protein family, is required for efficient inner kinetochore assembly in the pathogenic yeast Candida albicans. Mol. Microbiol. 80:14–32.

32. Duan Z, Androne scu M, Schutz K, McIlwain S, Kim YJ, Lee C, Shen-
dure J, Fields S, Blau CA, Noble WS. 2010. A three-dimensional model of the yeast genome. Nature 465:363–367.

33. Magee BB, Magee PT. 1997. WO-2, a stable aneuploid derivative of Candida albicans strain WO-1, can switch from white to opaque and form hyphae. Microbiology 143:289–295.

34. Thakur J, Sanyal K. 2013. Efficient neocentromere formation is sup-
pressed by gene conversion to maintain centromere function at native physical chromosomal loci in Candida albicans. Genome Res. 23:638–652.

35. Amor DJ, Bentley K, Ryan J, Perry J, Wong L, Slater H, Choo KHA. 2004. Human centromere repositioning “in progress.” Proc. Natl. Acad. Sci. U. S. A. 101:6542–6547.

36. Ravi M, Chan SWL. 2010. Haploid plants produced by centromere-mediated genome elimination. Nature 464:615–618.

37. Gillum A, Tsay E, Kirsch D. 1984. Isolation of the Candida albicans gene for orotidine-5’-phosphate decarboxylase by complementation of S. cerevisiae ura3 and E. coli pyrF mutations. Mol. Gen. Genet. 198:179–182.

38. Morschhäuser J, Ruhnke M, Michel S, Hacker J. 1999. Identification of CARE-2-negative Candida albicans isolates as Candida dubliniensis. Mycoses 42:29–32.

39. Stalb P, Moran GP, Sullivan DJ, Coleman DC, Morschhäuser J. 2001. Isogenic strain construction and gene targeting in Candida dubliniensis. J. Bacteriol. 183:2859–2865.

40. Wilson RB, Davis D, Mitchell AP. 1999. Rapid hypothesis testing with Candida albicans through gene disruption with short homology regions. J. Bacteriol. 181:1868–1874.

41. Strauss A, Michel S, Morschhäuser J. 2001. Analysis of phase-specific gene expression at the single-cell level in the white-opaque switching system of Candida albicans. J. Bacteriol. 183:3761–3769.

42. Negredo A, Monteilova L, Gil C, Pla J, Nombela C. 1997. Cloning, analysis and one-step disruption of the ARBS6 gene of Candida albicans. Microbiology 143:297–302.