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Genetics of the White-Opaque Transition in *Candida albicans*: Demonstration of Switching Recessivity and Mapping of Switching Genes

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Spheroplast fusion has been used to analyze the genetics of the reversible phenotypic transition, white-opaque, in *Candida albicans* WO-1. This transition involves changes in cell shape, permeability, and colony morphology. Fusion of switching with nonswitching cells gave nonswitching fusants, suggesting that the white-opaque phenotype is recessive. Chromosome loss induced by heat shock gave segregants of the fusants which were able to undergo the transition, indicating that the repressor function is genetically defined and probably limited to one or two chromosomes. Chromosomes R, 1, 3, 4, and 7 were eliminated as unique sites for the repressor, leaving 2, 5, and 6 as possible locations. When a *ura3* (chromosome 3) nonswitching strain was fused with a switching strain, all *ura3* segregants induced by heat shock were incapable of the phenotypic transition. Therefore, some or all of the genes (called *SWI* genes) essential for the transition are located on chromosome 3. UV irradiation-induced recombination did give rise to Ura⁻ switching progeny, showing that the failure to switch was not due to a side effect of the pyrimidine requirement. The failure to isolate normally switching *ura3* progeny generated by UV irradiation suggests a close linkage between the two genes.

Candida albicans is a pathogenic yeast which has become, since the advent of aggressive cytotoxic chemotherapy for cancer and organ transplant patients, a significant clinical problem. The organism is diploid as commonly isolated, and no sexual cycle has been observed for it (23). Among its interesting biological properties are its ability, induced by various culture conditions, to shift back and forth between a yeast and a hyphal form (the dimorphic transition) and its phenotypic instability, or change in colony morphology at a high frequency in an apparently stochastic manner.

The latter property was noted 20 years ago in the literature (9, 23, 28), but its significance was not appreciated until Slutsky et al. (24) showed that the colony phenotype of a particular strain, 3153A, changed in a complicated manner at a frequency too high to be the result of mutation. Later, the same laboratory reported a second form of phenotypic switching, the white-opaque transition (25). The white-opaque transition was first identified by the observation that strain WO-1 gave rise to two colony phenotypes; microscopic examination revealed that the cells in those two kinds of colonies had different shapes (Fig. 1). White cells are shaped like normal *C. albicans* yeast cells; opaque cells are slipper shaped and bud at an angle from one pole; they also grow more slowly than do white cells (25). The two kinds of cells differ in UV sensitivity, permeability, and many other properties (1–3, 19a, 20). Scanning electron microscopy indicates that the cell surface is altered and that the dynamics of budding are changed in opaque cells (3). The transition from opaque to white was shown to occur at about 5×10^{-4} per cell division at 24°C, while the reverse change occurs with a similar frequency in liquid YEPD medium. At 37°C, on the other hand, the white-to-opaque change appears not to occur; 10 h of incubation of an opaque culture at 37°C gives rise to 100% white colonies. This is not a selection, since the plating efficiency of opaque cells at this temperature is close to 100% (20). A single-cell study on solid defined medium gave a similar rate for white to opaque but a much higher opaque-to-white rate (4). The capacity to undergo the white-opaque transition has been found in several clinical isolates (26) and may be associated with virulence, although no evidence has yet been provided that WO-1, the original white-opaque isolate, is particularly pathogenic (19).

Two media have been devised to allow easy distinction between colonies that consist primarily of white cells and those predominantly opaque. These media allow one to carry out genetic experiments, since colonies that contain cells undergoing the transition are easily distinguishable from those that do not. One medium contains phloxin red (3); the other contains bismuth and is called BiBAGY medium. Opaque colonies turn brown on this medium; white cells are tan or white (20).

Since no sexual cycle is known in *C. albicans*, parasexual genetics must be used for mapping and complementation in this organism (for a review, see reference 23). This system of genetic analysis is relatively well developed in *C. albicans*; it consists of spheroplast fusion of strains containing complementary markers. Some of the heterokaryons originally formed undergo nuclear fusion to form tetraploids; others undergo limited genetic transfer from one nucleus to the other, leading to aneuploid strains. UV irradiation can be used to induce mitotic crossing over for linkage studies. Surprisingly, mitotic recombination seems to work quite well in tetraploids, even though they may be +/+/−/− for the allele in question (18). Alternatively, limited heat shock can induce chromosome loss, leading after several cycles to a return to the diploid state. Studies by Barton and Gull (3a) suggest that aneuploid strains (2N-1) may be able to survive, although they grow quite badly. On the other hand, extensive efforts with heterozygous diploids to induce auxotrophs by heat shock have failed (19a, 23). Thus, in mapping studies, the appearance of an auxotroph after heat shock of a heterozygous prototrophic fusant most likely signals that

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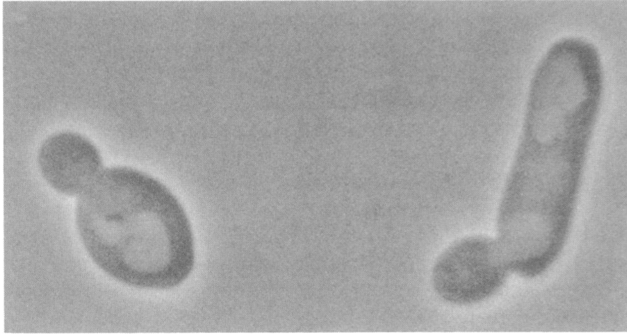

FIG. 1. White and opaque cells. Note the elongated cell shape and the characteristic budding angle of the opaque cell.

both of the homologs of the original auxotrophic parent are present.

To carry out a genetic analysis of the phenomenon of phenotypic instability, we generated auxotrophic mutants of WO-1 and fused them to SGY269, a *ura3* strain. By heat shock-induced chromosome loss and UV-induced recombination, we have been able to show that the white-opaque switch is recessive in the fusants, that switching segregants will arise after chromosome loss, and that some or all of the genes required for the transition to the opaque phenotype are located on chromosome 3, the linkage group containing *URA3*. Our results suggest that the recessive switching phenotype is under negative control. They also indicate that one or more of the genes essential for this phenotypic transition is on chromosome 3.

MATERIALS AND METHODS

Strains. Strain WO-1 was kindly provided by D. R. Soll. WO-1.3 is a His$^-$ derivative of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG)-treated WO-1. Subsequent NTG mutagenesis of WO-1.3 gave rise to NTG-1 (His$^-$ Arg$^-$), NTG-12 (His$^-$ Pro$^-$), and NTG-29 (His$^-$ Ilv$^-$). WO-1.14 (His$^-$ Arg$^+$) and WO-1.24 (His$^+$ Arg$^-$) were UV revertants of NTG-1. Sfo55 and Sfo81 were prototrophic derivatives of WO-1 which were altered in switching frequency. The origins of strains SGY269 (12) and hOG24 (18) have been described previously. Neither undergoes the white-opaque transition on YEPD medium or on BiBAGY medium. Strains 1006 and 1161 were provided by S. Scherer. Table 1 describes the strains used in this study.

Media. Strains were grown in YEPD medium (6) except where indicated otherwise. Auxotrophic requirements were analyzed on minimal medium supplemented with nutritional requirements according to standard procedures described by Fink (6). The composition and preparation of BiBAGY (bismuth) medium have been described previously (20).

Protoplast formation, fusion, and regeneration. The methods described by Poulter and Rikkerink (18) for *C. albicans* were used. DNA concentrations (Table 2) were determined as described by Block et al. (5), using a fluorescence-activated cell sorter.

Heat shock of fusion hybrids. Fusion products were heat shocked according to the protocol of Hilton et al. (8). Unless stated otherwise, a standard heat shock of 51.5°C for 90 s was used. Figure 2 shows how heat shock can be used to demonstrate both linkage and nonlinkage of genes. A fusant is made of a Gal$^-$ Ura$^+$ Ade$^+$ and a Gal$^+$ Ura$^-$ Ade$^-$ strain. The fusant is prototrophic. When subjected to heat shock, the fusant loses chromosomes in a random manner. Some segregants (I) will lose only the two homologs containing the *GAL1* gene; these will be Gal$^-$ but Ura$^+$ Ade$^+$. Other segregants (II) will lose the chromosomal homologs carrying the *URA3* gene. These will be Ura$^-$, but since *ADE2* is linked to *URA3*, and since one parent was Ura$^-$ Ade$^-$, most Ura$^-$ segregants will be Ade$^-$ as well. Thus, Ura$^-$ and Ade$^-$ appear together with a high frequency, while Ura$^-$ and Gal$^-$ do not. However, since more than one set of homologs can be lost in one heat shock exposure, some segregants will lose homologs for both chromosome 1 and chromosome 3 (III). These will be Gal$^-$ Ura$^-$ Ade$^-$. This rules out the possibility that Gal$^-$ and Ura$^-$ are linked but carried in *trans* in the original fusion.

Electrophoretic karyotype. The contour-clamped homogeneous electric field (CHEF) electrophoresis karyotype of various strains was determined as described by Magee et al. (16).

Preparation and digestion of DNA and separation of fragments. The methods described by Magee et al. (15) were used. Gel-fractionated DNA restriction fragments were transferred to a Zeta membrane or nitrocellulose filter by the methods of Southern (27).

RESULTS

The capacity to undergo the phenotypic transition is recessive. To make fusions between a white-opaque strain and a wild-type (nonswitching) strain, we needed to have auxotrophic derivatives of WO-1. UV irradiation, which stimulates switching, gave rise among the survivors to several auxotrophs, but all reverted at too high a frequency to make them useful. NTG, however, gave several mutants, including one which was His$^-$. Subsequent treatment with NTG

TABLE 1. Strains used

Name	Phenotypic transition	Auxotrophic marker(s)	Reference or source
WO-1	+	None	25
NTG-1	+	His$^-$ Arg$^-$	WO-1 (this study)
NTG-29	+	His$^-$ Ilv$^-$	WO-1 (this study)
WO-1.14	+	His$^-$	NTG-1 (this study)
WO-1.24	+	Arg$^-$	NTG-1 (this study)
SGY269	−	Ura$^-$ (*ura3*::*ADE2*/*ura3*::*ADE2*)	9
hOG24	−	Ade$^-$ Pro$^-$ Met$^-$	14
1006	−	MPA^r Ser$^-$ Ura$^-$ Lys$^-$ Arg$^-$	4
1161	−	MPA^r Gal$^-$ Ser$^-$ Ura$^-$ Lys$^-$ Arg$^-$	S. Scherer
Sfo55	Altered in frequency	None	WO-1 (this study)
Sfo81	Altered in frequency	None	WO-1 (this study)

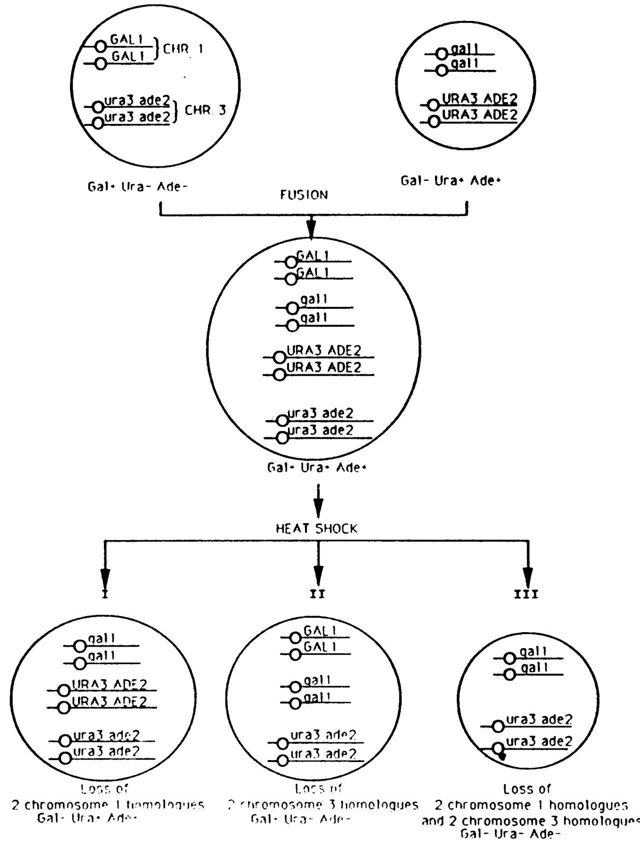

FIG. 2. Mapping by heat shock-induced loss of chromosomes. In this example, a Gal$^-$ strain is fused with a Ura$^-$ Ade$^-$ strain. The fusant is prototrophic. Heat shock leads to the appearance of either parental type (I, II) or a recombinant (III). The fact that there are no Ura$^-$ strains which are not Ade$^-$ and vice versa indicates that *URA3* and *ADE2* are linked. The fact that Gal$^-$ can occur in Ura$^+$ or Ura$^-$ segregants indicates that *GAL1* and *URA3* are unlinked.

gave NTG-1, which is His$^-$ Arg$^-$. Among the NTG-induced auxotrophs, several switched at lower or higher frequencies than did WO-1, although NTG-1 was in the normal range. NTG-1 was subjected to UV irradiation and plated on minimal-histidine medium and on minimal-arginine medium to select WO-1.14 (His$^-$ Arg$^+$) and WO-1.24 (His$^+$ Arg$^-$).

Each of these auxotrophs was fused with SGY269, a *ura3* strain. In addition, WO-1.14 was fused with hOG24 (Ade$^-$ Pro$^-$ Met$^-$). Several of the switching strains were fused to each other to ensure that the process of spheroplast fusion did not affect the phenotypic transition. Stable prototrophic colonies were picked. All of the fusants having one nonswitching parent grew uniquely as yeastlike (white) cells, as monitored by colony morphology on YEPD and BiBAGY and by phase microscopy, but when both parents were capable of the phenotypic transition, the fusants switched (Table 2). There are at least two possible explanations for this: either the white-opaque phenotype is recessive, or in the process of isolating the fusant, some entity carrying the switching capacity was lost. It has been documented that fusion hybrids of *C. albicans* sometimes result from unidirectional transfer of genetic material, leading to aneuploidy (7, 11, 22). Such an event could explain the failure of switching fusants to appear, although it would seem to be unlikely to occur every time. Furthermore, the DNA content of the fusants (Table 2) suggests that most are at least tetraploid. However, the recessivity hypothesis is easily testable. Heat shock at 51°C causes chromosome loss in fusion hybrids, so we treated several (nonswitching) fusants to induce chromosome loss and monitored the progeny for switching by plating on BiBAGY medium. Switching was monitored by the appearance of dark (opaque) or sectored light and dark colonies. In both cases, opaque cells appeared among the segregants at a frequency within an order of magnitude of that found for auxotroph segregants (Table 3). (In many heat shock experiments using fusions made from two nonswitching parents, we have never seen any white-opaque switching progeny.) This appearance suggests that there is a small number (one or two) of elements in the nonswitching strains that suppress the switching phenotype. Since this element can be lost by heat shock at a frequency similar to the appearance of auxotrophs, it is probably located on one of the chromosomes. We will call this function a repressor, although we have no idea of its molecular nature. In any case, the heat shock experiments suggest that the white-opaque phenotype is under negative control.

TABLE 2. Phenotypic transitions in fusion products of *C. albicans*

Strain	Transition	Relative DNA content
SCMS-7[a]	−	1.0
NTG-1	+	1.04
NTG-29	+	0.99
WO-1.14	+	1.10
WO-1.24	+	1.08
SGY269	−	1.55
hOG24	−	1.12
WO-1.14 × SGY29	−	2.74
NTG-1 × hOG24	−	2.25
WO-1.24 × SGY269	−	2.51
WO-1.14 × WO-1.24	+	2.42
NTG-1 × NTG-29	+	2.25

[a] Diploid *Saccharomyces cerevisiae*.

Chromosome 3 carries genes required for the white-opaque transition but does not carry the repressor function. *C. albicans* has eight chromosomes (10, 14, 21, 29). Auxotrophic markers have been localized to five of them. *URA3* is known to lie on chromosome 3 (16), and several *URA3*-containing plasmids which replicate in *C. albicans* are known (13). We therefore set out to construct a Ura$^-$ strain capable of the phenotypic transition in order to be able to clone the genes involved in this process. We attempted to make *ura3* switching strains by heat shock from a nonswitching fusant of WO-1.14 × SGY269 and WO-1.24 × SGY269. We hoped to isolate strains which had lost both the repressor

TABLE 3. Production by heat shock of auxotrophs and white-opaque segregants from fusants

Fusions[a]	No. of colonies examined	No. of auxotrophs	No. of white-opaque colonies
WO-1.14 (S) × SGY269 (NS)	22,000	18	3
	5,600	25	5
WO-1.24 (S) × SGY269 (NS)	6,600	20	12
NTG-1 (S) × hOG24 (NS)	10,000	15	1

[a] S, switcher; NS nonswitcher.

TABLE 4. Phenotypes of fusants and segregants from fusions of switching and nonswitching strains[a]

Parents	No. of switchers	No. of auxotrophs	Auxotrophic segregants[b]
NTG-1 × hOG24	1	15	4 Ade$^-$ Met$^-$ (1) 2 Pro$^-$ (0) 9 His$^-$ Arg$^-$ (0)
WO-1.14 × SGY269	3[c]	18[d]	6 His$^-$ (1) 11 Ura$^-$ (0)
	5[c]	25[d]	8 His$^-$ (2) 16 Ura$^-$ (0)
WO-1.24 × SGY269	12[c]	20	1 Arg$^-$ (0) 19 Ura$^-$ (0)

[a] Phenotypes of all fusants were stable; all cells were subjected to heat shock.
[b] Number of switchers of each auxotrophic type is given in parentheses.
[c] Prototrophic switchers account for the difference between this number and the number of auxotrophic segregants.
[d] One auxotroph with unknown requirements was isolated.

function and the *URA3*-bearing homologs of chromosome 3. Of 46 Ura$^-$ segregants, however, none had gained the ability to undergo the phenotypic transition (Table 4). The heat shock treatment also gave rise to some prototrophic switching segregants. One of the prototrophic switching segregants, HSO(2)-1, was then heat shocked again. We isolated 22 Ura$^-$ segregants, all of which had lost the ability to undergo the phenotypic transition (Table 5). When we used UV instead of heat shock, 52 of the 83 Ura$^-$ segregants from HSO(2)-1, or 63%, could switch (Table 5). We interpret this to mean that one or more genes essential to the white-opaque phenotypic transition are located on chromosome 3 in WO-1.14 and that when this chromosome is lost, the cells are unable to switch. We call this gene(s) *SWI*, for switching. The fact that *ura3* switching strains can be isolated after UV-induced mitotic recombination suggests that the *SWI* gene(s) is centromere proximal. Since the original switching segregant contained the SGY269 chromosome 3 homologs, as shown by the fact that we were able to get Ura$^-$ segregants from it, we can eliminate chromosome 3 as the location for the repressor function (see below).

SGY269 was constructed by insertion of a piece of DNA containing the *ADE2* gene into the *URA3* gene (12). It is conceivable that this insertion might affect recombination of the adjacent *SWI* gene(s). Furthermore, WO-1.14 and WO-1.24 have karyotypic variations which seem to have been induced by the mutagenesis used to isolate them. We therefore fused Sfo81, a prototrophic derivative of the original white-opaque strain, with 1006, a Lys$^-$ Ser$^-$ Arg$^-$ *ura3* strain which is also resistant to mycophenolic acid (MPA) and which does not switch on BiBAGY, YEPD, or low-zinc medium (4). Fusants of strain 1006 with a prototrophic strain can be selected on the basis of the dominant resistance to the inhibitor MPA. Fusants of Sfo81 and 1006 behaved exactly as did the fusants mentioned above, except that UV irradiation of Swi$^+$ progeny heterozygous for *ura3* yielded only five switchers out of 30 Ura$^-$ (Table 5). These results show that for a completely separate fusion, the *SWI* gene(s) maps to chromosome 3 and that the repressor is not on that linkage group. Interestingly, none of the Ura$^-$ white-opaque strains switches normally. Some switch less frequently, some switch more frequently, and some show an abnormal response to temperature.

WO-1 carries a small extra chromosome of about 700 kb which we have referred to as the supernumerary chromosome (*snc*) (20). It seemed possible that this chromosome carried some genes essential to switching. McEachern and Hicks (17) have presented evidence that loss of this chromosome depresses the switching frequency. We examined the karyotypes of several white-opaque segregants of the cross WO1.14 × SGY269 and found two where the *snc* was lost. Figure 3 shows that in HS9 and HSO(2)-1, two switching

TABLE 5. Segregants of prototrophic white-opaque (switching) fusants derived by heat shock of nonswitching fusants

Strain	Parents	Treatment	No. of switchers	No. of auxotrophs	Auxotrophic segregants[a]
HSO(2)-1	WO-1.14 × SGY269(2)	HS	0	22	22 Ura$^-$ (0)
		UV	58[b]	85	83 Ura$^-$ (52) 2 His$^-$ (1)
HSO(3)	1006 × Sfo81	UV	1	10	10 Ura$^-$ (1)
HSO(4)	1006 × Sfo81	UV	0	4	4 Ura$^-$ (0)
HSO(5)	1006 × Sfo81	UV	3	12	12 Ura$^-$ (3)
HSO(6)	1006 × Sfo81	UV	6	9	5 Lys$^-$ (5) 4 Ura$^-$ (1)
HSO(7)	1006 × Sfo55	UV	17	23	10 Lys$^-$ (10) 13 Arg$^-$ (7)
HSO(8)	1006 × Sfo55	UV	5	7	5 Ura$^-$ (4) 2 Lys$^-$ (1)
HSO(9)	hOG24 × NTG1	HS	1	6	6 Ade$^-$ (1)
HSO(11)	1161 × NTG-1	HS	70	139	33 Gal$^-$ (17) 21 Gal$^-$ Lys$^-$ (10) 7 Gal$^-$ Lys$^-$ His$^-$ (6) 3 Gal$^-$ Lys$^-$ His$^-$ Ura$^-$ (0) 4 Gal$^-$ His$^-$ (3) 1 Gal$^-$ Ura$^-$ (0) 1 His$^-$ (1) 1 Ura$^-$ (0) 66 Lys$^-$ (32) 2 Lys$^-$ His$^-$ (1)

[a] Number of switchers of each auxotrophic class is given in parentheses.
[b] Prototrophic switchers account for the difference between this number and the number of auxotrophic segregants.

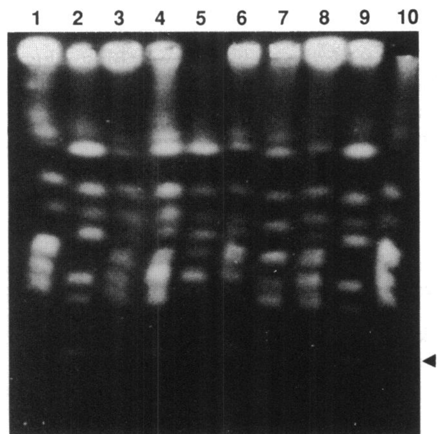

FIG. 3. CHEF chromosome separation. Fusion parents, fusants, and segregants were subjected to CHEF pulsed-field separation as described in Materials and Methods. Lanes: 1 and 10, SGY269; 2 and 9, WO-1.14; 3 and 8, WO-1.14 × SGY269; 4, HSO(1)-1; 5, HS9; 6, HS23; 7, HSO(2)-1. HSO(1)-1, HS9, HS23, and HSO(2)-1 are switching segregants of WO-1.14 × SYG269. The arrow points to the position of the *snc* (~700 kb).

heat shock segregants of WO-1.14 × SGY269, the *snc* band is missing. It is thus clear that the *snc* is not required for switching. The *snc* is composed of fragments of chromosome 5 and chromosome 6 (5a), and its loss in the diploid WO-1 may depress switching either because of unbalanced gene dosage for those chromosomes or because of the changes associated with the reorganization of the genome (e.g., endoreduplication of other copies of chromosome 5 and 6) to compensate for its loss.

The repressor of the phenotypic transition lies on chromosome 2, 5, or 6. We also set out to try to map the repression phenotype to one of the bands in the karyotype. The approach we used involved eliminating chromosomes as a site for the repressor on the basis of their retention in a switching segregant derived from a nonswitching fusant.

Chromosomes were identified by auxotrophic markers. The appearance of an auxotrophic marker from the nonswitching (wild-type) parent in a switching segregant demonstrated the presence of the corresponding chromosome and eliminated it as a site for the repressor. Since the organism apparently must retain two copies of each chromosome, appearance of auxotrophy requires that both homologs from the nonswitching strain be present. Heterozygosity for the repressor in the nonswitching strain is thereby controlled for.

Chromosome R. Chromosome R contains the rDNA gene in *C. albicans* as well as the *ADE1* gene (29). Tables 4 and 5 show that a fusant and a segregant from a cross of NTG-1 × hOG24 (*ade1/ade1*) gave white-opaque Ade$^-$ segregants. In another experiment, red (Ade$^-$) auxotrophs isolated by heat shock from the same cross gave three Ade$^-$ switchers upon further heat shock. This rules out chromosome R as the site for the repressor.

Chromosome 1. Chromosome 1 contains the marker *GAL1* (16). Table 5 shows that in HSO(11), a switching strain derived by heat shock from a fusant of strains NTG-1 and 1161 (*ser57/ser57 gal1/gal1 lys1/lys1 arg57/arg57*), Gal$^-$ segregants arise after heat shock. Thus, chromosome 1 is eliminated as the site for the repressor, since both homologs from the nonswitching parent must have been present in HSO(11).

Chromosome 4. The *lys1* gene is located on chromosome 4 in 1161 (23). Thus, chromosome 4 is eliminated, since Lys$^-$ switchers occurred in segregants from HSO(6), HSO(7), HSO(8), and HSO(11) (Table 5).

Chromosome 7. The *arg57* gene is on chromosome 7 (23). From a fusion of 1006 with Sfo55, a white-opaque segregant, HSO(7), was isolated. It gave rise to Arg$^-$ auxotrophs, eliminating chromosome 7 as the repressor site (Table 5).

With the elimination of five of the eight *C. albicans* chromosomes as the unique site for the repressor, we are left with chromosomes 2, 5, and 6 as possible locations. None of these chromosomes has a genetic marker useful for these studies. We therefore cannot eliminate any as a potential locus for the repressor function at this time.

DISCUSSION

The mechanistic basis for the phenotypic transition in *C. albicans* is not understood. This organism has been shown to have a variety of unstable phenotypes which change with a frequency much greater than mutation could account for. These phenotypes include colony morphology (24) and cell shape (25), as well as a variety of less easily observed properties. It is by no means certain that the mechanism leading to these changes is always the same, but it seems likely to be the case. We have therefore begun to investigate the genetics of the white-opaque transition in the hope that any information that we generate will be of general use in understanding the phenomenon. Although parasexual genetics is cumbersome, even when we use molecular techniques to speed the process, we have been able to demonstrate two important facts about the white-opaque transition: it is recessive, and genes necessary for the process are on chromosome 3, closely linked to *URA3*. By the process, we mean, of course, the appearance of the opaque phenotype with a high frequency. The change from white to opaque must be quite complicated, and any of the steps involving either the switch or the appearance of the phenotype could be coded for on chromosome 3 or by the target of the suppression. The evidence that the white-opaque phenotypic transition is recessive agrees with the observation of Goshorn and Scherer (7), who found that a colony morphology transition was recessive. The similarity in the two systems renders less likely, but does not rule out, the possibility that recessivity is at the level of the white-opaque phenotype, not the switch itself. A formal genetic description would say that there are two dominant genes (the repressor and the switching gene), with the former epistatic to the latter. However, in the absence of more information, it is easiest to think about the process as repression. Whether the recessive nature of the transition is due to a classical repressor or not, it is evident that the mechanism of repression is relatively simple, since the genes required for it must be confined to one or a few chromosomes. This conclusion is based on the fact that switching segregants appear from nonswitching fusants at a frequency from 5 to 50% of that of total auxotrophs. If there were many loci with the possibility of repressing switching, one would expect the frequency to be very much lower. If repression were a complex phenomenon, requiring many unlinked genes, one would expect the frequency to be much higher. Thus, a single locus model fits the data best.

One interpretation of the recessive nature of the white-opaque transition would be that the stability of the majority of *Candida* isolates is due to repression of switching genes which are universally present. This interpretation is rendered less likely by the observation that strain SGY269 lacks

at least some of the genes for switching carried on chromosome 3 in WO-1.14. This is shown by the fact that loss of chromosome 3 of WO-1.14, revealing the *ura3* phenotype of SGY269, invariably leads to a loss of switching. Thus, SGY269 appears to carry the repressor but not the switching genes that it represses. On the other hand, 1006, a strain which suppresses WO-1 switching when it is a parent in fusions (Table 5), will spontaneously yield white-opaque progeny at a very low frequency (5a). Whether the lack of the switching genes in SGY269 or the potential for the transition found in 1006 is the rule for nonswitching strains cannot be decided from our data.

The nature of the repressor is quite unclear. Although it is simplest to discuss this function as though it were encoded by a single gene, we have no evidence on this point. With the auxotrophic strains presently available, we have been able to eliminate only five of the eight chromosomes as its site. Ideally, one would like to show cosegregation of repression with an auxotrophic marker and thus map the function positively. For this we need more markers. The fact that the repressor is not present in WO-1 and that it is present in all nonswitching strains tested suggests that it may have functions other than the repression of the phenotypic transition; indeed, repression may be the side effect of some metabolic function which is missing in WO-1. Alternatively, the repressor may be multifunctional in most strains but have lost one of its functions by mutation in WO-1. Precisely mapping the repressor will allow us to design experiments that might rule out one of these alternatives.

The linkage of *URA3* and the switching gene is fortuitous. The fact that we have failed to isolate a *ura3* auxotroph which switches normally suggests relatively close linkage of the two functions, but we do not know what close linkage means in *C. albicans* in terms of base pairs. The *URA3* gene has been cloned and is the most commonly used marker for *C. albicans* transformation. If necessary, it could be used as a base for chromosome walking experiments to look for the sequences involved in the phenotypic transition. The cloning will be simplified by the fact that in the absence of the repressor, at most only two copies of the *SWI* gene are required to convey the transition phenotype. This is evident from the fact that UV-induced mitotic crossing over gives Ura^- Swi^+ progeny, from a switching Ura^+ parent. The genotype of the parent must then be *ura3/ura3/URA3/URA3 SWI/SWI/swi/swi*, and the progeny are, of course, homozygous for *ura3* and probably *swi/swi/SWI/SWI*. Thus, in both parent and fusant, the presence of the *SWI* gene(s) on two of the four chromosome 3 homologs leads to a transition phenotype. Another possible route to the Ura^- switching strains would be UV-induced mitotic crossing over followed by chromosome loss. In this case, the progeny would be *swi/swi/SWI*. Thus, in the absence of repressor, the switching phenotype must be dominant over the nonswitching phenotype.

This preliminary genetic analysis of the phenotypic transition has allowed us to define some important properties of the system, opening the way to a molecular analysis of the problem. It seems clear that some genes relative to the transition can be cloned, since Ura^- nonswitchers can be generated by heat shock- or UV-induced mitotic crossing over from strains which are competent to switch. Furthermore, genetic and molecular analysis of the repressor function can be carried out. Since Goshorn and Scherer have shown that colony morphology transitions are recessive (7), it would be interesting to know whether the same function regulates the white-opaque transition as controls colony morphology switching. A genetic approach is perhaps the best way to test the possibility. Evaluation of the similarities and differences of the various switching phenotypes will allow us both to understand this interesting biological phenomenon and to evaluate its role in the pathogenesis of *Candida* infections.

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REFERENCES

1. **Anderson, J. M., L. Cundiff, B. Schnars, M. Gao, I. Mackenzie, and D. R. Soll.** 1989. Hypha formation in the white-opaque transition of *Candida albicans*. Infect. Immun. **57:**458–467.
2. **Anderson, J. M., R. Mihalik, and D. R. Soll.** 1990. Ultrastructure and antigenicity of the unique cell wall "pimple" of the *Candida* opaque phenotype. J. Bacteriol. **172:**224–235.
3. **Anderson, J. M., and D. R. Soll.** 1987. Unique phenotype of opaque cells in the white-opaque transition of *Candida albicans*. J. Bacteriol. **169:**5579–5588.

3a. **Barton, R., and K. Gull.** 1992. Isolation, characterization, and genetic analysis of monosomic aneuploid mutants of *Candida albicans*. Mol. Microbiol. **6:**171–177.

4. **Bergen, M. S., E. Voss, and D. R. Soll.** 1990. Switching at the cellular level in the white-opaque transition of *Candida albicans*. J. Gen. Microbiol. **136:**1925–1036.
5. **Block, D. E., P. D. Eitzman, J. D. Wangensteen, and F. Srienc.** 1990. Slit scanning of *Saccharomyces cerevisiae* cells: quantification of asymmetric cell division and cell cycle progression in asynchronous culture. Biotechnol. Prog. **6:**504–512.

5a. **Chu, W.-S.** Unpublished data.

5b. **Chu, W.-S., and P. T. Magee.** Unpublished data.

6. **Fink, G. R.** 1970. The biochemical genetics of yeast. Methods Enzymol. **17:**59–78.
7. **Goshorn, A. K., and S. Scherer.** 1989. Genetic analysis of protrophic natural variants of *Candida albicans*. Genetics **123:** 617–673.
8. **Hilton, C., D. Markie, B. Corner, E. Rikkerink, and R. Poulter.** 1985. Heat-shock induced chromosome loss in the yeast *Candida albicans*. Mol. Gen. Genet. **200:**162–168.
9. **Ireland, R., and A. Sarachek.** 1969. Introduction and selection of the minute-rough (MR) colony variant of *Candida albicans*. Mycopathologica **37:**377–392.
10. **Iwaguchi, S.-I., M. Homma, and K. Tanaka.** 1990. Variation in the electrophoretic karyotype analysed by the assignment of DNA probes in *Candida albicans*. J. Gen. Microbiol. **136:**2433–2442.
11. **Kakar, S. N., R. M. Partridge, and P. T. Magee.** 1983. A genetic analysis of *Candida albicans*: isolation of a wide variety of auxotrophs and demonstration of linkage and complementation. Genetics **104:**241–255.
12. **Kelly, R., S. M. Miller, M. B. Kurtz, and D. R. Kirsch.** 1987. Directed mutagenesis in *Candida albicans*: one-step gene disruption to isolated *ura3* mutants. Mol. Cell. Biol. **7:**199–207.
13. **Kurtz, M. B., M. W. Cortelyou, S. M. Miller, M. Lai, and D. R. Kirsch.** 1987. Development of autonomously replicating plasmids for *Candida albicans*. Mol. Cell. Biol. **7:**209–217.
14. **Lasker, B. A., G. F. Carle, G. S. Kobayashi, and G. Medoff.** 1989. Comparison of the separation of *Candida albicans* chromosome-sized DNA by pulsed-field gel electrophoresis techniques. Nucleic Acids Res. **17:**3783–3793.
15. **Magee, B. B., T. M. D'Souza, and P. T. Magee.** 1987. Strain and species identification by restriction fragment length polymorphisms in the ribosomal DNA repeat of *Candida* species. J. Bacteriol. **169:**1639–1643.
16. **Magee, B. B., Y. Koltin, J. Gorman, and P. T. Magee.** 1988.

Assignment of cloned *Candida albicans* genes to the seven electrophoretically separated chromosomes. Mol. Cell. Biol. **8:**4721–4726.

17. **McEachern, M. J., and J. Hicks.** 1991. Dosage of the smallest chromosome affects both the yeast-hyphal transition and the white-opaque transition of *Candida albicans* WO-1. J. Bacteriol. **173:**7436–7442.
18. **Poulter, R., and E. Rikkerink.** 1983. Genetic analysis of red, adenine-requiring mutants of *Candida albicans*. J. Bacteriol. **156:**1066–1077.
19. **Ray, T. L., and C. D. Payne.** 1990. *Candida albicans* acid proteinase: a role in virulence, p. 163–178. *In* E. M. Ayoub, G. H. Cassell, W. C. Branche, Jr., and T. J. Henry (ed.), Microbial determinants of virulence and host response. American Society for Microbiology, Washington, D.C.

19a. **Rikkerink, E. H. A.** Unpublished data.

20. **Rikkerink, E. H. A., B. B. Magee, and P. T. Magee.** 1988. Opaque-white phenotypic transition: a programmed morphological transition in *Candida albicans*. J. Bacteriol. **170:**895–899.
21. **Rustechenko-Bulgac, E. P., F. Sherman, and J. B. Hicks.** 1990. Chromosomal rearrangements associated with morphological mutants provide a means for genetic variation in *Candida albicans*. J. Bacteriol. **169:**4393–4395.
22. **Sarachek, A., and D. A. Weber.** 1984. Temperature-dependent internuclear transfer of genetic material in heterokaryosis of *Candida albicans*. Curr. Genet. **8:**181–187.
23. **Scherer, S., and P. T. Magee.** 1991. Genetics of *Candida albicans*. Microbiol. Rev. **54:**226–241.
24. **Slutsky, B., J. Buffo, and D. R. Soll.** 1985. High-frequency switching of colony morphology in *Candida albicans*. Science **230:**666–669.
25. **Slutsky, B., M. Staebell, J. Anderson, L. Risen, M. Pfaller, and D. R. Soll.** 1987. "White-opaque transition": a second high-frequency switching system in *Candida albicans*. J. Bacteriol. **169:**189–197.
26. **Soll, D. R., C. J. Langtimm, J. McDowell, J. Hicks, and R. Galosk.** 1987. High-frequency switching in *Candida* strains from vaginitis patients. J. Clin. Microbiol. **25:**1611–1622.
27. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
28. **Vogel, R. A., and R. S. Sponcler.** 1970. The study and significance of colony dissociation in *Candida albicans*. Sabouraudia **7:**273–278.
29. **Wickes, B. L., J. Staudinger, B. B. Magee, K.-J. Kwon-Chung, P. T. Magee, and S. Scherer.** 1991. Physical and genetic mapping of *Candida albicans*: several genes previously assigned to chromosome 1 map to chromosome R, the rDNA-containing linkage group. Infect. Immun. **59:**2480–2484.