Genetic Analysis of Prototrophic Natural Variants of *Candida albicans*

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Manuscript received May 10, 1989
Accepted for publication August 17, 1989

ABSTRACT

To facilitate genetic analysis of *Candida albicans* natural variants, we have isolated a dominant mycophenolic acid-resistant mutant. Mycophenolic acid-resistant auxotrophs were used to analyze prototrophic natural variants by spheroplast fusion. The fusion products were shown to be heterozygous for many of the parental chromosomes by molecular and genetic criteria. Using this approach, we have found that one type of morphologic variation is due to a recessive change and identified three dominant 5-fluorocytosine-resistant mutants. Rare fusion products express recessive parental markers. These exceptional progeny should be useful for linkage analysis and strain construction.

CANDIDA albicans is one of the most common fungal pathogens. Molecular analysis has shown that Candida infections are caused by a great diversity of strains. They vary in drug resistance (DEFEVER *et al.* 1982), virulence in animal models (KWON-CHUNG *et al.* 1985), and many other phenotypic characteristics. Natural isolates often differ in colony morphology (SLUTSKY, BOFFO and SOLL 1985) and certain strains reversibly vary their morphology at high frequency (SLUTSKY *et al.* 1987).

Genetic analysis of these phenomena is greatly complicated by the absence of a known sexual cycle for this generally diploid yeast. A parasexual genetic system has been developed for this species that employs spheroplast fusion for complementation testing (KAKAR, PARTRIDGE and MAGEE 1983; POULTER *et al.* 1981), and chromosome loss for linkage determination (HILTON *et al.* 1985). Selection of fusion products has relied on complementation of auxotrophies in both parent strains; therefore, clinical strains have had to be mutagenized before such analyses could be done. Many strains yield auxotrophs only after severe mutagenesis and have significant changes in virulence (SHEPHERD 1985; MANNING, SNODDY and FROMTLING 1984).

Dominant markers would be of great value for the genetic analysis of *C. albicans* prototrophs and for the DNA transformation system (KURTZ, KIRSCH and KELLY 1988). Direct selection for spontaneous drug resistance mutations would be less likely to affect virulence; however, Candida is resistant to many antibiotics used with other fungi (SHEPHERD, POULTER and SULLIVAN 1985) and there is considerable variability among isolates in their resistance to commonly used agents.

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We describe here *C. albicans* strains that contain a dominant mutation for mycophenolic acid resistance, along with several auxotrophic markers. These strains can be used for spheroplast fusion with a great variety of unmarked clinical strains. Analysis of the fusion products has enabled us to make preliminary genetic characterizations of two phenotypes in one of these strains. We show that these methods produce a high degree of heterozygosity for parental markers, by both physical and genetic criteria. Rare fusion products reveal recessive parental markers. These exceptional progeny should facilitate strain construction and genetic mapping in *C. albicans*.

MATERIALS AND METHODS

Growth and mutant isolation: The compositions of rich medium (YPD) and yeast minimal medium (MIN) have been described (SHERMAN, FINK and LAWRENCE 1978). Required supplements were added to give a final concentration of 100 μg/ml. Mycophenolic acid (MPA) was added to MIN agar to give a final concentration of 5 μg/ml unless otherwise indicated. 5-Fluorocytosine (5-FC) was used at 30 μg/ml or 50 μg/ml in MIN agar. The test for sensitivity to 5-FC was the same as that of WHELAN, MARKIE and KWON-CHUNG (1986). A sensitive control strain was always included on the same plate for comparison. Cultures and plates were grown at 30°. The method for *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment has been described (POULTER and RIKKERINK 1983). For the 1,2,7,8-diepoxyoctane treatment, exponential phase cells were washed twice with water, and resuspended in 9.7 ml of 0.2 M potassium phosphate (pH 7.5), 2% glucose. 0.3 ml of 1,2,7,8-diepoxyoctane (Aldrich Chemical Company, Milwaukee, Wisconsin) was added. The cells were incubated at 30° with gentle shaking for 5 min, immediately washed and resuspended in YPD, and incubated for 2 hr more at 30° with gentle shaking. They were plated on YPD, grown for 2 days, and then replica plated onto appropriately supplemented MIN to identify mutants.

Strains: The *C. albicans* strains involved in this study are listed in Table 1. Strain 981 was a spontaneous MPA resistant mutant of strain 834. Strain 1006 was isolated from strain 981 by 1,2,7,8-diepoxyoctane mutagenesis as a Ura$^-$

auxotroph. It was shown to be *ura3* by complementation with the cloned *URA3* gene (KURTZ *et al.* 1987) using DNA transformation. The plasmid pMK22, which contains the *C. albicans URA3* gene was kindly provided by ROSEMARIE KELLY. Strains 1033 and 1034 are independent spontaneous 5-FC resistant mutants of 981. Strain 984 was isolated from strain 609 by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis of strain 609 as a red adenine auxotroph. It was shown to be *ade2* by complementation of the *ade1* mutation in spheroplast fusion to strain 982 and failure to complement the *ade2* mutation in spheroplast fusion to strain 833.

Spheroplast fusion: The protocol was modified from that of POULTER *et al.* (1981). All incubations were 30° for 30 min. The spheroplasts were washed twice with 0.5 M $CaCl_2$, 0.1 M Tris-hydrochloride (pH 7.2), and resuspended in 0.5 ml of the same buffer. In fusions of MPA-resistant strains to wild-type strains, spheroplasts were combined in a 7:1 ratio (Mpa^R: Mpa^S), and the progeny were selected on MPA-MIN. Fusion products typically took 7–10 days to appear on MPA-MIN.

Heat shock: Heat shock treatment was performed by the method of HILTON *et al.* (1985).

UV irradiation: UV treatment was similar to that of WHELAN and MAGEE (1981). The UV dose rate at the agar surface was 37 ergs/mm^2/sec, and spread plates were irradiated for 18 to 25 sec, depending on the strain, aiming for 10% survival. (Fusion products were less sensitive to UV than the normal diploid strains.)

DNA isolation, agarose gels and hybridizations: The procedure for small scale DNA isolations for *C. albicans* has been described (SCHERER and STEVENS 1987). Agarose gel electrophoresis, Southern transfers, and hybridizations with nick-translated probes were performed as described by SCHERER and STEVENS (1988).

Ribosomal DNA cloning: The ribosomal DNA probe was isolated as follows. A *Sal*I library of DNA from strain 616 (Table 1) was prepared in lambda vector EMBL3. Plaques were screened by hybridization to ^{32}P-labeled 616 DNA. Plaques that strongly hybridized were purified, and *Sal*I digests of the phage DNA rescreened by Southern analysis with a ^{32}P-labeled clone of the *S. cerevisiae* rDNA repeat. A clone that strongly hybridized to the *S. cerevisiae* rDNA was selected. The insert was a 12.1-kb *Sal*I fragment, which is the same size as the intensely staining band in a *Sal*I digest of total genomic *C. albicans* DNA.

RESULTS

MPA is an inhibitor of IMP dehydrogenase, and hence blocks *de novo* synthesis of GMP (FRANKLIN and COOK 1969; LEE *et al.* 1985). The growth of all *C. albicans* strains we examined was inhibited by 5–10 μg/ml MPA, and resistant mutants were rare. This inhibition is completely reversed by addition of guanine or guanosine to the medium.

We isolated a spontaneous mutant of the triply auxotrophic strain 834 (Table 1), called 981, that is resistant to at least 50 μg/ml MPA. The dominant nature of this MPA resistance mutation was determined as follows. MPA-sensitive segregants were isolated from strain 981 by UV-induced mitotic recombination (see MATERIALS AND METHODS). The sensitive colonies were found at a frequency of about 0.1% per UV survivor. This is the same as the frequency observed by WHELAN and MAGEE (1981) for segregation of auxotrophies from naturally heterozygous isolates. Strain 981 was fused to several auxotrophic strains and prototrophic fusion products were selected. The fusion products were both large fast growing colonies, and more numerous small slow growing colonies, as seen previously (POULTER *et al.* 1981; KAKAR, PARTRIDGE and MAGEE 1983). In all cases, the fast growing fusion products were MPA resistant and the MPA-resistant phenotype was stable after passage in nonselective medium. The slow growing colonies were sensitive to MPA and are discussed below. The MPA resistant fusion products were treated by heat shock to induce chromosome loss. Again, sensitive colonies arose at the expected frequency (about 1%), demonstrating heterozygosity for MPA resistance of the fusion products.

Strain 981 (Arg^- Ser^- Lys^- Mpa^R) was fused to strain 984, a red *ade2* mutant. The products were selected on minimal medium (MIN). Again, fusion products appeared as large white colonies on MIN with a background of smaller, pink colonies. The large white colonies were all MPA resistant. The smaller pink colonies were MPA sensitive and unstable for prototrophy, segregating white and red sectors. The pink colonies also occasionally gave rise to MPA resistant prototrophic segregants. These pink colonies therefore have the expected behavior of heterokaryons (POULTER *et al.* 1981). MPA resistance, while dominant in mononucleate cells, appears recessive in heterokaryons.

MPA resistance can be directly selected in spheroplast fusion experiments, despite the apparent sensitivity of the heterokaryons. The fusion products took 5–6 days longer to appear compared with selection for prototrophy, but the background of small colonies was significantly reduced. Similar results were obtained with strain 1006, a 1,2,7,8-diepoxyoctane induced *ura3* mutant of strain 981.

Fusions to wild-type strains: Fusion products between the MPA-resistant auxotrophs and six unrelated wild-type strains (Table 1) were selected directly on minimal medium containing 5 μg/ml MPA (MPA-MIN). 609, 616 and 666 were representative clinical isolates. Strain 987 overproduces extracellular protease, and has been shown to be highly virulent in a mouse model (KWON-CHUNG *et al.* 1985). Strains 655 and 985 are colony morphology variants.

We found that MPA-resistant fusion products could be obtained most efficiently by mixing spheroplasts in a ratio of 7:1 (Mpa^R:Mpa^S). This appears to reduce background growth of the wild-type parent. Occasionally, "escaper" colonies arose after prolonged incubation when spheroplasts of the wild-type strains were plated on MPA-MIN, but they did not grow when transferred to fresh MPA-MIN, distinguishing them from true fusion products. For the wild-type strains

TABLE 1

***C. albicans* strains**

Strain	Synonym	Relevant characteristics	Source/Reference
Auxotrophs			
834	STN57	Arg$^-$ Ser$^-$ Lys$^-$	SUZUKI, ROGERS and MAGEE (1986)
981		Arg$^-$ Ser$^-$ Lys$^-$ Mpa^R	This work
1006		Arg$^-$ Ser$^-$ Lys$^-$ *ura3* Mpa^R	This work
1033		Arg$^-$ Ser$^-$ Lys-5–FC resistant	This work
1034		Arg$^-$ Ser$^-$ Lys-5–FC resistant	This work
984		*ade2*	This work
833	hOG300-4	*ade2 pro MET/met*	E. RIKKERINK
982	hOG24	*ade1 pro met*	POULTER and RIKKERINK (1983)
Prototrophs			
666		Wildtype	D. STEVENS
655		Wrinkled colonies, 5-FC resistant	D. STEVENS
987	C9	Virulent in mice	KWON-CHUNG *et al.* (1985)
985	WO-1	White-opaque transition	SLUTSKY *et al.* (1987)
609		Wildtype	SCHERER and STEVENS (1988)
616		Wildtype, Type 2 rDNA	SCHERER and STEVENS (1988)

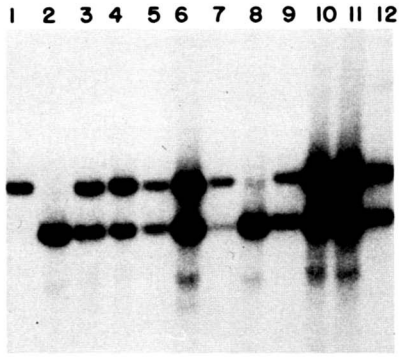

FIGURE 1.—rDNA heterozygosity of 981 × 655. DNA from 981 (lane 1), 655 (lane 2), and ten independent 981 × 655 fusion products (lanes 3–12) was digested with *Hin*fI, and hybridized with the *C. albicans* rDNA probe.

985 and 616, which were less sensitive to MPA, fusion products were selected on 10 μg/ml MPA, to further reduce this background. No prototrophic revertants of 981 or 1006 were detected.

Heterozygosity for parental markers: DNA from the fusion products was examined to determine whether it contained chromosomes from both parent strains. The *C. albicans* ribosomal DNA (rDNA) is located on chromosome *I* (MAGEE *et al.* 1988). Restriction fragment length polymorphisms are easily detected in the rDNA by Southern analysis of chromosomal DNA digested with *Eco*RI or *Hin*fI, which cut the tandem repeats (MAGEE, D'SOUZA and MAGEE 1987).

Figure 1 shows a Southern analysis of fusion products from 981 × 655 using a ^{32}P-labeled *C. albicans* rDNA clone (see MATERIALS AND METHODS). Fusion products usually contained the rDNA-hybridizing *Hin*fI fragments from both parents, indicating that they were heterozygous for chromosome *I*. The two bands in the heterozygous fusion products can have unequal intensities (lanes 7 and 9), so fusion products

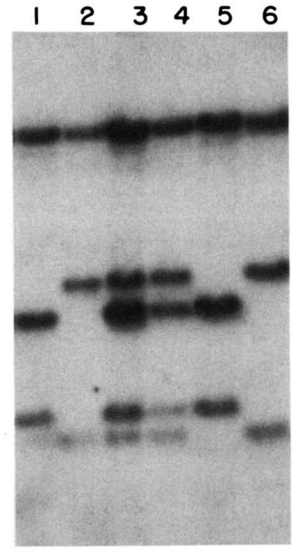

FIGURE 2.—rDNA heterozygosity of 981 × 616. DNA from 981 (lane 1), 616 (lane 2), and four 981 × 616 fusion products (lanes 3–6) was digested with *Eco*RI and hybridized with the *C. albicans* rDNA probe.

may contain variable numbers of the parental chromosomes. A similar analysis of fusion products from 981 × 616 is shown in Figure 2. 616 shows the rarer type 2 rDNA banding pattern in an *Eco*RI digest, seen in about 10% of clinical isolates (SCHERER and STEVENS 1987). The fusion products in lanes 3 and 4 contain the hybridizing fragments from both parents, while the fusion products in lanes 5 and 6 show only the rDNA bands from 981 and 616, respectively. It is important to note that the fusion product in lane 6 contains copies of chromosome *I* from 616 only. If the chromosomes are inherited intact in spheroplast fusion, the MPA resistance mutation cannot be located on chromosome *I*. Strains such as those in lane 6 could arise by mutation of 616 to MPA resistance, rather than fusion with 981. We believe this unlikely

TABLE 2

Frequency of ribosomal DNA heterozygosity in fusion products

Fusion	Heterozygous	Mpa^R parent only	Mpa^S parent only	Total
981 × 609	2	1	0	3
981 × 616	3	2	2	7
981 × 987	2	0	0	2
981 × 666	0	0	3	3
981 × 985	3	0	0	3
981 × 655	12	1	1	14
Total	22	4	6	32

The values represent the pooled data from several separate fusion experiments. Southern analyses were performed as described in Figures 1 and 2.

based on the low frequency of spontaneous MPA resistant mutants and the absence of resistant colonies when 616 spheroplasts are plated on MPA-MIN. Also, rDNA patterns characteristic only of the sensitive parent are seen in fusion products with strain 984 (*ade2*, see above) after selecting for MPA resistant prototrophs. In this case, mutation to MPA resistance and reversion of the auxotrophy would be required to yield such a product by a mechanism other than fusion.

A summary of the rDNA heterozygosity of fusion products is shown in Table 2. The most common fusion products are heterozygous for chromosome *I* (22 out of 32), but fusion products that are homozygous for either parental chromosome *I* are also found. Since several of the prototrophs have the 981 chromosome *I* only, we propose that none of the auxotrophies can map to chromosome *I*, if chromosomes are inherited intact. The *arg*, *ser*, *lys* and *ura3* mutations are known to be unlinked to each other by heat shock analyses (E. RIKKERINK, personal communication).

Heterozygosity for chromosomes other than chromosome *I* was determined by segregation of parental markers from the fusion products. Chromosome loss was achieved either by passage in nonselective medium or by heat shock. Segregants were scored by replica plating from YPD master plates onto MPA-MIN plates. Isolation of MPA sensitive segregants from fusion products, as described above, indicates that the MPA sensitivity allele from the unmarked parent was present in the original fusion product. Arg^-, Ser^-, Lys^- and Ura^- segregants all were isolated from products of 981 and 1006 fusions to prototrophs. In two cases, multiple different single auxotrophs were recovered from the same fusion product. The fusion products appear to be heterozygous for many unselected chromosomes. A more detailed analysis of heterozygosity for chromosome *III* is described below.

Phenotypes in natural variants: We have examined the utility of MPA-resistant strains as test strains for genetic analysis of unusual phenotypes of unmarked, wild-type strains. Strain 655 makes colonies that wrinkle on agar, while 981 and 1006 make conventional smooth colonies (Figure 3). MPA resistant prototrophs were selected from 981 × 655 and 1006 × 655 fusions. They were streaked for single colonies on MPA plates, and then examined on YPD to test for the parental phenotypes. Both parental phenotypes were found among the fusion products (Table 3), as well as two additional ones; one phenotype in which all of the colonies in the streak were uniformly wrinkled but less so than 655 (intermediate), and another phenotype that was unstable, and showed a mixture of smooth, and variably wrinkled colonies. The two new phenotypes seen in the fusion products have also been observed in clinical isolates (not shown). As seen in Table 3, the majority of fusion products fell into the "mixed" category. The 655-like morphology was observed in only one fusion product out of 34, suggesting that wrinkled is recessive to smooth.

Strain 655 is also resistant to 5-FC. 655 was fused to 1006, selecting MPA-resistant prototrophs, and these were retested on 5-FC. Table 4 shows that the majority of the MPA-resistant fusion products were also resistant to 5-FC, so the 5-FC resistance determinant in 655 appears to behave as a dominant allele.

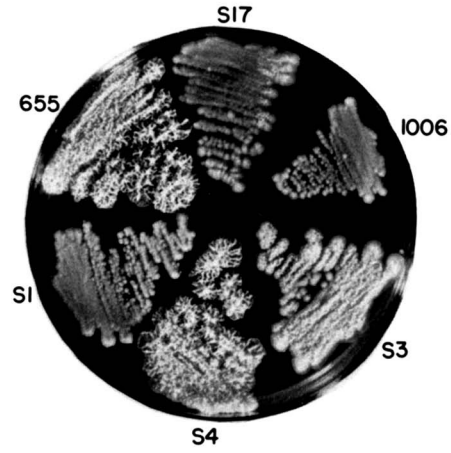

FIGURE 3.—Colony morphology phenotypes of 1006 × 655. The parent strains 1006 (smooth) and 655 (wrinkled) are shown, along with four 1006 × 655 fusion products. S3 shows the "intermediate" phenotype described in the text and Table 3, while S1 and S17 have "mixed" phenotypes. The S4 phenotype is identical to that of 655.

TABLE 3

Colony morphology phenotypes from fusions to 655

	Phenotype				
Fusion	Like 981 (smooth)	Like 655 (wrinkled)	Intermediate	Mixed	Total
981 × 655	5	0	4	10	19
1006 × 655	3	1	4	7	15
Total	8	1	8	17	34

TABLE 4

Fusions to 5-FC-resistant strains

Fusion	Total Screened	5-FC^R
1033 × 666	106	98 (92%)
1034 × 666	138	126 (91%)
1006 × 655[a]	78	65 (83%)

[a] For this fusion, only Ura$^+$ progeny were scored for 5-FC resistance.

Previously described 5-FC-resistant mutants in *C. albicans* have been recessive (WHELAN, MARKIE and KWON-CHUNG 1986). 5-FC-resistant 655 × 981 fusion products were treated by heat shock to check for heterozygosity of the 5-FC resistance locus by chromosome loss. 5-FC-sensitive segregants from these fusion products were readily obtained.

We also isolated two independent, spontaneous 5-FC-resistant mutants of 981, 1033 and 1034, and fused them to the 5-FC-sensitive, prototrophic strain, 666. MPA-resistant prototrophs were selected, and tested for 5-FC resistance. In both cases, more than 90% of the MPA-resistant fusion products were also resistant to 5-FC (Table 4). To confirm that 5-FC resistance in our mutants was dominant, we treated 1033 and 1034 with UV light to see if the sensitive phenotype could be segregated. Both 1033 and 1034 yielded 5-FC-sensitive segregants, supporting the view that they are heterozygous for dominant mutations.

Exceptional progeny: We had previously observed that MPA-resistant fusion products could segregate individual parental auxotrophies, even without heat shock treatment. In our experiments in which 981 or 1006 were fused to wild-type strains, fusion products were routinely selected on MPA-MIN. Any progeny that did not contain chromosomes to complement each auxotrophy would not have been detected. We define exceptional progeny as fusion products that do not display all dominant parental markers.

The quadruple auxotroph, 1006, was fused to two unmarked strains, 655 and 666. Fusion products were not selected on MPA-MIN but on MPA-MIN plus two of the four required supplements for 1006. All six combinations of two supplements were tested. Fusion products selected on these media were tested on MPA-MIN. Those that grew on the supplemented media, and not on MPA-MIN, were analyzed.

Table 5 lists the exceptional progeny seen in this experiment. Most of the fusion products are prototrophs and probably have a nearly complete set of wild-type chromosomes. In both fusions, single auxotrophs outnumbered double auxotrophs among the exceptional progeny. A total of 17 Lys, 8 Arg, 9 Ser and 22 Ura auxotrophs were obtained. Combining the data from the two fusions, all of the possible double auxotrophs were found.

To determine if MPA enhances chromosome loss

TABLE 5

Exceptional progeny from fusions to wild type

Markers scored	1006[a] × 666[b] Auxotrophs Double	1006[a] × 666[b] Auxotrophs Single[c]	1006[a] × 666[b] Total tested	1006 × 655[b] Auxotrophs Double	1006 × 655[b] Auxotrophs Single[c]	1006 × 655[b] Total tested
arg ser	0	0	30	2	2	60
arg lys	2	2	60	0	3	42
arg ura	1	3	40	0	1	60
ser lys	0	1	35	1	4	60
ser ura	2	2	43	1	3	60
lys ura	1	9	60	1	4	60
Total	6	17	278	5	17	342

[a] The phenotype of 1006 is Arg$^-$ Ser$^-$ Lys$^-$ Ura$^-$ Mpa^R.
[b] 666 and 655 are prototrophs.
[c] The values in the single auxotrophs column represent the total number of single auxotrophs for the two markers tested.

TABLE 6

Exceptional progeny from 1006 (Arg$^-$ Ser$^-$ Lys$^-$ *ura3* Mpa^R) × 984 (*ade2*)

Selection	Number Tested	Exceptions
Minimal	120	1 Mpa^S
Minimal + uridine	120	1 Mpa^S
MPA + adenine	120	4 Ade$^-$
MPA + uridine	120	2 Ura$^-$

in fusion products, and if there is any bias as to which parental chromosome is discarded, the following experiment was performed. 1006, which has a *ura3* mutation on chromosome *III*, was fused to 984, which has an *ade2* mutation on chromosome *III* (MAGEE *et al.* 1988). Fusion products from 1006 × 984 were selected on four different media (Table 6). These were patched onto the same media, grown, and replica plated onto MPA-MIN plates and MIN plates, to identify the exceptional progeny.

The results in Table 6 show that when MPA is included in the selective medium, there is at most a small effect on the number of exceptions. We did not observe a large difference in Ura$^-$ *versus* Ade$^-$ exceptional progeny, comparing the MPA + uridine selection with the MPA + adenine selection. Again, if chromosomes are inherited intact, the isolation of MPA-resistant *ade2* exceptions excludes *MPA1* from chromosome *III*. These results show that most fusion products are heterozygous for chromosome *III* markers.

DISCUSSION

We have identified a dominant drug resistance mutation in *C. albicans*, *MPA1*, and demonstrated its use as a selectable marker in spheroplast fusion studies. This approach permits direct genetic analysis of *C. albicans* clinical isolates, without prior mutagenesis.

Selection on media containing MPA appears to

exclude heterokaryons, which are often observed in spheroplast fusion experiments. A similar genetic behavior is seen with the *KAR1* gene of *S. cerevisiae*. Even though wild-type *KAR1* is dominant and the *KAR1* product is made in the cytoplasm, the *KAR1* gene behaves as a recessive during mating (DUTCHER and HARTWELL 1983).

The fusion products that we have analyzed are largely heterozygous for the parental markers. There is an approximately equal likelihood for markers on chromosomes of either parent to be absent in an exceptional fusion product. Since rDNA, *arg*, *ser*, *lys*, and *ura3* are unlinked, a prototrophic MPA resistant fusion product of 1006 × wild type can easily be shown to be heterozygous for five of the seven *C. albicans* chromosomes by recovery of the auxotrophies and by analysis of RFLPs at chromosome *I*. Alignment of the physical and genetic maps and identification of additional markers will extend this type of analysis to the remaining *C. albicans* chromosomes. In fusions where markers for a particular chromosome are not selected, exceptional progeny can be easily identified. The recovery of exceptional progeny, as seen by physical and genetic criteria, demonstrates that all of the fusion products generated in a given cross are not heterozygous for every chromosome.

There are a number of ways that the exceptional progeny might arise from spheroplast fusions. The spontaneous appearance of auxotrophs from initially prototrophic fusion products suggests that fusion of the nuclei results in a tetraploid fusion product. Then, in the absence of selection for a particular marker, chromosomes could be discarded or segregate aberrantly, resulting in the exception. A prediction of this model is that singly auxotrophic exceptions would occur more often than doubly auxotrophic exceptions. This agrees with our observations (Table 5).

An alternate mechanism for generation of exceptional progeny would be transfer of a subset of chromosomes from one nucleus to another, without karyogamy (KAKAR, PARTRIDGE and MAGEE 1983). This effect occurs in *KAR1* × *kar1* in *S. cerevisiae* (DUTCHER 1981). This mechanism clearly does not operate in the majority of fusion events. If it did, the high percentage of progeny heterozygous for unselected chromosomes would not be seen; however, the exceptions may not arise by the same mechanism as the fusion products where all markers are selected.

The observation of excess single over double auxotrophs in Table 5 is consistent with previous heat-shock studies (E. RIKKERINK, personal communication), which showed that the *arg*, *ser*, *lys* and *ura3* mutations are unlinked. This experiment also suggests a new method to construct *C. albicans* strains. By controlling which markers are selected in a fusion and which are not, one can obtain strains that have a particular set of unlinked markers in a single step, rather than relying on the two-step fusion, chromosome loss procedure.

Our analysis of the wrinkled phenotype of 655 revealed fusion products that did not resemble either parent. The most common fusion product (Table 3), gave rise to a mixture of different morphologic variants when a single colony was streaked. This could be explained by spontaneous loss of unselected chromosomes in the fusion products. We suspect that the fusion products may contain unequal amounts of the parental chromosomes, and the relative gene dosage from each parent may explain their variable levels of expression. We consider wrinkled to be the recessive phenotype, because the wrinkled parental phenotype was seen in only one fusion product out of 34, while the smooth parental phenotype was more prevalent.

The dominant 5-FC resistance that we have observed in strains 1033, 1034, and 655 has not been reported in *C. albicans*. WHELAN and KERRIDGE (1984) have characterized clinical *C. albicans* strains that were "partially resistant" to 5-FC. These strains grew slowly on 5-FC medium, while highiy resistant strains grew normally and sensitive strains did not grow. Upon mitotic recombination, the partially resistant strains gave rise to sensitive and highiy resistant cosegregants indicating that partial resistance was due to heterozygosity for recessive mutations. Two complementing alleles, *fcy1* and *fcy2*, were identified, which correlated with reduced enzyme levels of UMP pyrophosphorylase and cytosine deaminase, respectively (WHELAN, MARKIE and KWON-CHUNG 1986). Dominant 5-FC resistance mutations at the FUR1, FUR2 (URA2), and FUR4 loci have been described in *S. cerevisiae* (JUND and LACROUTE 1970). For the three 5-FC resistant strains we examined, the overwhelming majority of MPA resistant fusion products were also 5-FC resistant. Fusion to 981 or 1006 selecting for MPA-resistant prototrophs should permit rapid screening of natural variants for dominant mutations, but final assurance of dominance relations will require segregation of the alternate phenotype.

Spheroplast fusion analyses using a multiply marked strain such as 1006 could be useful for mapping of new recessive mutations isolated in that background. Recovery of the mutant phenotype with a particular auxotrophy in a majority of the exceptional progeny in fusions to wild type would demonstrate genetic linkage.

The *ura3* mutation in 1006 makes the exceptional progeny useful for construction of hosts for DNA transformation. KURTZ *et al.* (1987) have described high frequency transformation vectors containing ARS sequences and the *C. albicans URA3* gene. The availability of such vectors, combined with the ability to rapidly construct suitable host strains, should facil-

itate the isolation of genes that control the many interesting Candida phenotypes seen in nature.

This work was supported by U.S. Public Health Service grant AI23850. We would like to thank P. T. MAGEE and E. RIKKERINK for helpful discussions, and P. T. MAGEE for suggestions on this manuscript.

LITERATURE CITED

DEFEVER, K. S., W. L. WHELAN, A. L. ROGERS, E. S. BENEKE, J. M. VESELENAK and D. R. SOLL, 1982 *Candida albicans* resistance to 5-fluorocytosine: frequency of partially resistant strains among clinical isolates. Antimicrob. Agents Chemother. **22:** 810–815.

DUTCHER, S. K., 1981 Transfer of genetic information in *kar1-1/KAR1* heterokaryons in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1:** 245–253.

DUTCHER, S. K., and L. H. HARTWELL, 1983 Genes that act before conjugation to prepare the *Saccharomyces cerevisiae* nucleus for caryogamy. Cell **33:** 203–210.

FRANKLIN, T. J., and J. M. COOK, 1969 The inhibition of nucleic acid synthesis by mycophenolic acid. Biochem. J. **113:** 515–524.

HILTON, C., D. MARRIE, B. CORNER, E. RIKKERINK and R. T. POULTER, 1985 Heat shock induces chromosome loss in the yeast *Candida albicans*. Mol. Gen. Genet. **200:** 162–168.

JUND, R., and F. LACROUTE, 1970 Genetic and physiological aspects of resistance to 5-fluoropyrimidines in *Saccharomyces cerevisiae*. J. Bacteriol. **102:** 607–615.

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.

KURTZ, M. B., D. R. KIRSCH and R. KELLY, 1988 The molecular genetics of *Candida albicans*. Microbiol. Sci. **5:** 58–63.

KURTZ, M. B. M. W. CORTELYOU, S. M. MILLER, M. LAI and D. R. KIRSH, 1987 Development of autonomously replicating plasmids for *Candida albicans*. Mol. Cell. Biol. **7:** 209–219.

KWON-CHUNG, K. J., D. LEHMAN, C. GOOD and P. T. MAGEE, 1985 Genetic evidence for role of extracellular protease in virulence of *Candida albicans*. Infect. Immun. **49:** 571–578.

LEE, H. J. K. PAWLAK, B. T. NGUYEN, R. K. ROBINS and W. SADEE, 1985 Biochemical differences among four inosinate dehydrogenase inhibitors, mycophenolic acid, ribavirin, tiazofurin, and selenazofurin, studied in mouse lymphoma culture. Cancer Res. **45:** 5512–5520.

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.

MAGEE, B. B., Y. KOLTIN, J. A. GORMAN and P. T. MAGEE, 1988 Assignment of cloned genes to the seven electrophoretically separated *Candida albicans* chromosomes. Mol. Cell. Biol. **8:** 4721–4726.

MANNING, M., C. B. SNODDY and R. A. FROMTLING, 1984 Comparative pathogenicity of auxotrophic mutants of *Candida albicans*. Can. J. Microbiol. **30:** 31–35.

POULTER, R. T., and E. H. RIKKERINK, 1983 Genetic analysis of red, adenine-requiring mutants of *Candida albicans*. J. Bacteriol. **156:** 1066–1067.

POULTER, R. K. JEFFERY, M. J. HUBBARD, M. G. SHEPHERD and P. A. SULLIVAN, 1981 Parasexual genetic analysis of *Candida albicans* by spheroplast fusion. J. Bacteriol. **146:** 833–840.

SCHERER, S., and D. A. STEVENS, 1987 Application of DNA typing methods to epidemiology and taxonomy of Candida species. J. Clin. Microbiol. **25:** 675–679.

SCHERER, S., and D. A. STEVENS, 1988 A *Candida albicans* dispersed, repeated gene family and its epidemiologic applications. Proc. Natl. Acad. Sci. USA **85:** 1452–1456.

SHEPHERD, M. G., 1985 Pathogenicity of morphological and auxotrophic mutants of *Candida albicans* in experimental infections. Infect. Immun. **50:** 541–544.

SHEPHERD, M. G., R. T. M. POULTER and P. A. SULLIVAN, 1985 *Candida albicans*: biology, genetics, and pathogenicity. Annu. Rev. Microbiol. **39:** 579–614.

SHERMAN, F., G. R. FINK and C. W. LAWRENCE, 1978 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

SLUTSKY, B., J. BUFFO and D. R. SOLL, 1985 High-frequency switching of colony morphology in *Candida albicans*. Science **230:** 666–669.

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:** 1889–1897.

SUZUKI, T., A. L. ROGERS AND P. T. MAGEE, 1986 Inter- and intra-specific crosses between *Candida albicans* and *Candida guillermondi*. Yeast **2:** 53–58.

WHELAN, W. L., and D. KERRIDGE, 1984 Decreased activity of UMP pyrophosphorylase associated with resistance to 5-fluorocytosine in *Candida albicans*. Antimicrob. Agents Chemother. **26:** 570–574.

WHELAN, W. L., and P. T. MAGEE, 1981 Natural heterozygosity in *Candida albicans*. J. Bacteriol. **145:** 896–903.

WHELAN, W. L., D. MARKIE and K. J. KWON-CHUNG, 1986 Complementation analysis of resistance to 5-fluorocytosine in *Candida albicans*. Antimicrob. Agents Chemother. **29:** 726–729.

Communicating editor: E. W. JONES