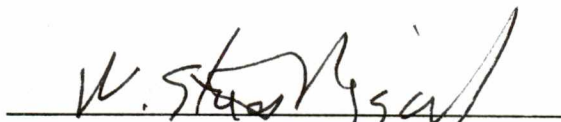


To the Graduate Council;

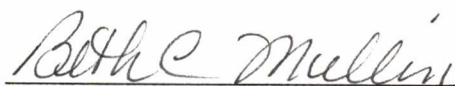
I am submitting herewith a dissertation written by Ronald Arthur Uphoff entitled "Genetic analysis of *Candida albicans* Germ Tube Mutants." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Microbiology.

  
W. Stuart Riggsby, Major Professor

We have read this dissertation  
and recommend its acceptance:







Accepted for the Council:

  
Associate Vice Chancellor and  
Dean of The Graduate School

GENETIC ANALYSIS OF  
*CANDIDA ALBICANS* GERM TUBE MUTANTS

A Dissertation  
Presented for the  
Doctor of Philosophy  
Degree  
The University of Tennessee, Knoxville

Ronald Arthur Uphoff  
May 1996

This dissertation is dedicated to the memory of Kailie Lynn Lee.

I wish to acknowledge the guidance and patience of my committee: Dr. Jeff Becker, Dr. Bert Lampson, and Dr. Beth Mullin. I also wish to acknowledge the time, patience, guidance and encouragement of my major professor, Dr. Stuart Riggsby.

For the gift of invaluable strains and vectors, I thank Alvin Sarachek, Stewart Scherer, Richard Cannon and B. B. Magee.

Most of all, I thank my wife Jennie and my whole family for their love and their unyielding faith that I would actually finish this thing.

## ABSTRACT

A spontaneous *Candida albicans* mutant incapable of forming germ tubes in serum was isolated from a doubly auxotrophic strain by successive rounds of filtration enrichment.

The above strain and three other independently derived germ tube mutants were tested for complementation of the mutant germ tube phenotypes by spheroplast fusion. The four mutants fell into three complementation groups.

The parasexual cycle was used to construct and analyze hybrids from a germ tube mutant or its derivatives, and two mapping strains. The analysis indicated that one mutant germ tube locus (*gtf1*) is on either chromosome 5 or 6. This analysis also revealed that two previously unmapped marker loci (*MPA1* and *SFI86*) are on chromosome 2.

During this work, it was discovered that UV irradiation, which is known to induce mitotic recombination, also induces random chromosome loss in *C. albicans* hybrids.

The parasexual cycle was also used to construct a *ura3 gtf1* strain which served as a host for a *C. albicans* genomic library in a *C. albicans* /*S. cerevisiae* /*E. coli* shuttle vector. The attempt to isolate a gene which complements the *gtf1* mutation was unsuccessful. However, the library was shown to contain single copy nuclear genes by the isolation of *PTR2*.

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## LIST OF ABBREVIATIONS

|       |                                              |
|-------|----------------------------------------------|
| AMP   | Ampicillin                                   |
| ATCC  | American Type Culture Collection             |
| BiGGY | Bismuth Glucose Glycine Yeast Extract Medium |
| C     | Celsius                                      |
| DNA   | Deoxyribonucleic Acid                        |
| dpm   | Disintegrations Per Minute                   |
| EDTA  | Disodium Ethylenediaminetetraacetic Acid     |
| EtdBr | Ethidium Bromide                             |
| IPTG  | Isopropyl-1-Thio- $\beta$ -D-Galactoside     |
| Kbp   | Kilobases                                    |
| LB    | Luria Bertani Medium                         |
| Mbp   | Megabases                                    |
| MPA   | Mycophenolic Acid                            |
| mtDNA | Mitochondrial DNA                            |
| n     | Haploid Genome                               |
| rDNA  | Ribosomal DNA                                |
| RFLP  | Restriction Fragment Length Polymorphism     |
| rpm   | Revolutions Per Minute                       |
| SD    | Synthetic Dextrose Medium                    |
| SDS   | Sodium Dodecyl Sulfate                       |
| SSC   | Sodium Chloride, Sodium Citrate Buffer       |
| SSV   | Starch Salts and Vitamins Medium             |
| STC   | Sorbitol Tris Calcium Chloride Buffer        |
| TAE   | Tris Acetate EDTA Buffer                     |

|         |                                                    |
|---------|----------------------------------------------------|
| TBE     | Tris Borate EDTA Buffer                            |
| TE      | Tris EDTA Buffer                                   |
| UV      | Ultraviolet Radiation                              |
| X-gal   | 5-Bromo-4-Chloro-3-Indolyl- $\beta$ -D-Galactoside |
| YPAD    | Yeast Extract Peptone Adenine Dextrose Medium      |
| YPD     | Yeast Extract Peptone Dextrose Medium              |
| $\mu$ g | Microgram                                          |
| $\mu$ l | Microliter                                         |

PART I  
GENERAL INTRODUCTION

## CHAPTER I

### INTRODUCTION

The dimorphic yeast *Candida albicans* is a commonly isolated human commensal which is capable of infecting almost all organs and systems (Odds, 1988). Usually, however, most cases of *C. albicans* infections involve superficial tissues such as mucosae and skin (Shepherd et al., 1985).

One of the most interesting features of *C. albicans* is its ability to convert between budding growth as a yeast and filamentous (hyphal) growth (figure 1). The label "dimorphic", though, is a convenient simplification. The shape of the yeast form can range from nearly spherical to a cigar-shaped pseudohypha that is often mistaken for a true hypha. Merson-Davies & Odds, (1989) present an excellent description of the various shapes and a solution to the morphological ambiguity in the form of a numerical index based on the ratio of length and septal junction diameter to maximum diameter. In spite of the first-glance similarity of true hyphae and pseudohyphae, there are qualitative and quantitative differences between the two habits. The most notable differences are described by McGinnis (1980) and Mitchell & Soll (1979, 1979a). Among these are the absence of constriction at the germ tube - mother cell junction, straight sides, and perpendicular septa. Syverson et al., (1975) and Casanova et al. (1989) have shown that different antigens can be detected in the two forms

A variety of substrates have been demonstrated to induce yeast cells to produce hyphae *in vitro*. The general requirements for germ tube (nascent hypha) formation are near neutral pH and 37° C incubation. Different investigators use different media for germ tube induction. The most reliable inducer is mammalian serum (Evans et al., 1974; Ogletree et al., 1978; Odds, 1988). The synthetic amino acid broth devised by Lee et al. (1975) has

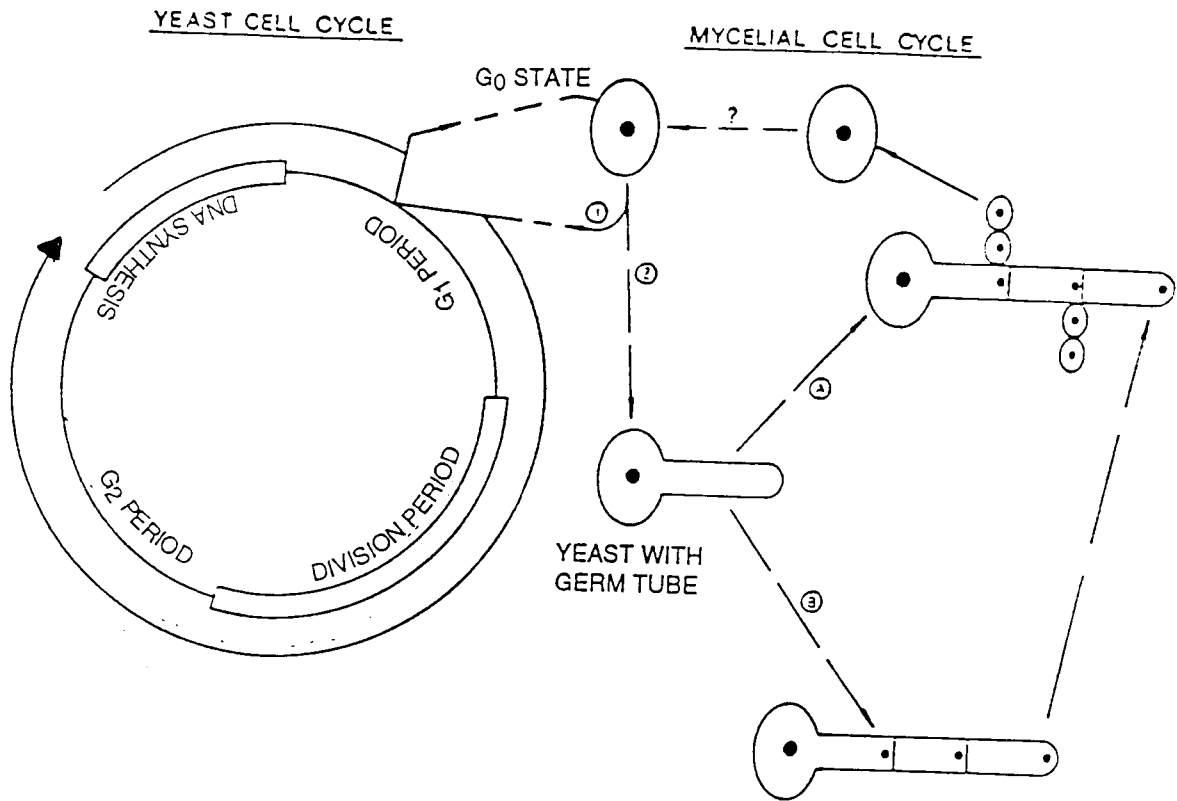


Figure 1. Scheme of the dimorphic cell cycle of *Candida albicans*. Symbols stand for: (1) transition to yeast cell cycle; (2) transition to yeast with germ tube; (A) transition to mycelial growth by hyphal lengthening only; arrows with broken lines refer to transitions related to mycelial cell cycle, and solid arrows refer to transitions related mainly to yeast cell cycle.

Torres-Bauza, 1980

been used in the extensive dimorphism research of Soll's group (Mitchell & Soll, 1979; Mitchell & Soll, 1979a; Soll et al., 1981; Anderson & Soll, 1984; Finney et al., 1985). Other inducers include starch (Marriott, 1975; Cannon, 1986; this work), *N*-acetylglucosamine (Simonetti et al., 1974 ; Shepherd et al., 1979) and L-proline (Ogletree et al., 1978). Figure 2 shows *Candida albicans* with germ tubes formed after 1.5 h of incubation in starch salts and vitamins medium (SSV) [panel A] and with hyphae and lateral buds after 5 h incubation in SSV [panel B]

An interesting but widely ignored exception to the requirement for neutral pH (6.5 - 7.0) is a report by Pollack & Hashimoto (1987). Their work suggests that in the absence of glucose, *C. albicans* can be induced to form germ tubes at pH 2.5, but the presence of glucose at a concentration as low as 200 mM inhibits germ tube formation below pH 6.0.

Although both the yeast and hyphal forms are found in infected tissue, the ability of *C. albicans* to produce hyphae is commonly regarded as a virulence factor,[ reviewed in Odds, (1988, 1994) and Gow, (1994)]. The hyphal form has been shown to adhere better than the yeast form to epithelial cells (Kimura & Pearsall, 1978; King et al., 1980; Sobel et al., 1981) , and Sobel et al., (1984) have shown that a mutant incapable of hyphal growth has a greatly reduced ability to establish vaginal infections in mice when compared to the germ tube-producing parent strain. The same germ tube-defective mutant was also shown to be less lethal in mice than the germ tube-producing parent or revertant administered at the same dosage (Buckley et al., 1986). Another hyphal mutant used in the studies of Ryley & Ryley, (1990) is pathogenic but does not invade the kidney as deeply as germ tube-producing strains. Evidence to support the notion that the role of hyphae is to penetrate tissue comes from the work of Sherwood et al., (1992) which demonstrates the contact sensing ability of *C. albicans* hyphae by their inclination to change direction to follow a groove or enter a pore in a filter on medium which encourages hyphal growth.

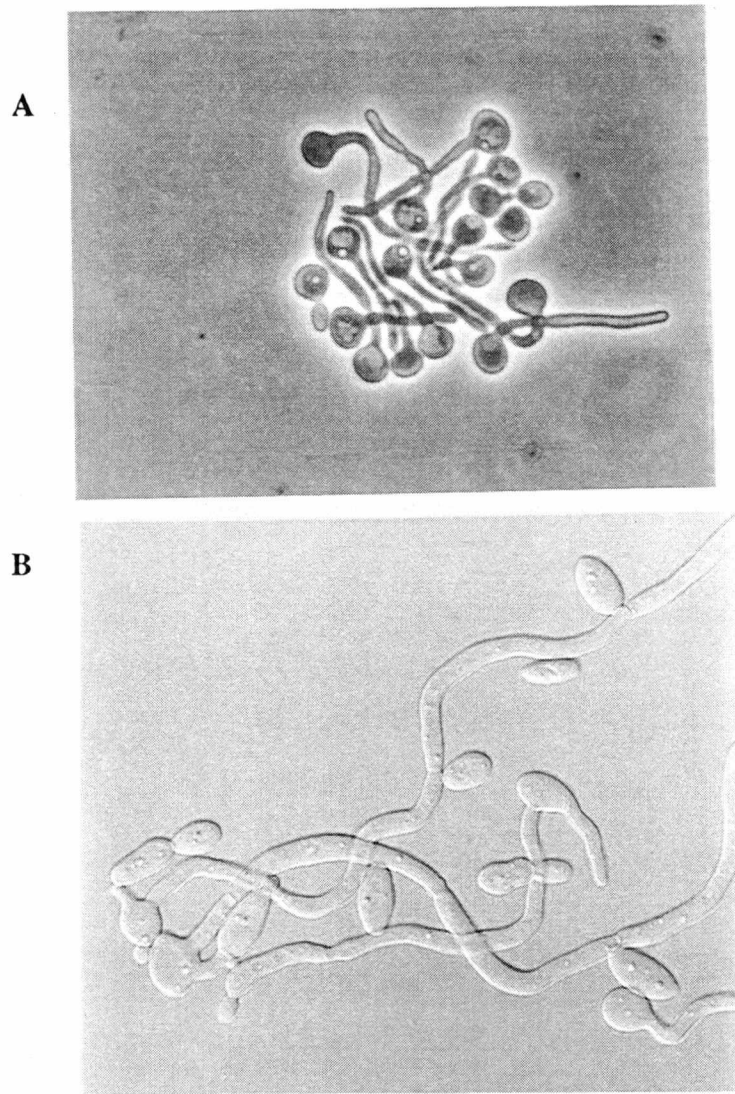


FIGURE 2. *Candida albicans* germ tubes and hyphae. Panel (A): Yeasts with germ tubes after incubation for 1.5 h in SSV at 37°C. Panel (B): Hyphae with laterally budding yeasts after incubation for 5 h in SSV at 37°C.

Although there is strong evidence to suggest that hyphal growth aids in the establishment of infection, it is also clear that the inability to exhibit hyphal growth does not preclude pathogenesis. In the studies cited above, the effect of hyphal deficiency on pathogenesis was a matter of degree; that is the mutant was shown to be *less* virulent rather than avirulent. In contrast to the prevailing view today, the formation of germ tubes by *C. albicans* was proposed by Reynolds & Braude, (1956) as the reason the organism was rarely found in systemic infections, in contrast to the yeast pathogens such as *Cryptococcus* and *Blastomyces* which are commonly associated with systemic infections. Although these *systemic* pathogens are capable of filamentous growth on culture media, only the yeast form is found in infected tissue.

It would not be surprising to find that, when *C. albicans* is incubated under conditions that favor hyphal growth, changes occur in gene expression and metabolic activity that are not necessarily involved directly with hyphal growth. Therefore, careful genetic analysis of hyphal mutants is fundamental to understanding the dimorphic transition between yeast and hyphal growth. However, genetic studies of *C. albicans* are impeded by the organism's diploid genome (Whelan & Magee, 1981; Riggsby et al., 1982) and absence of a sexual cycle. Parasexual genetics have been used with success to demonstrate allelic and nonallelic relationships among mutations (Kakar & Magee, 1982), and to demonstrate genetic linkages (Sarachek et al., 1981; Chu et al., 1992). Parasexual techniques can be applied to germ tube (hyphal) mutants to elucidate allelic relationships of the various mutations and to infer assignment of a mutation to a particular chromosome (linkage group).

Ideally, one would like to clone the genes required for germ tube formation. This would allow analysis of the inferred amino acid sequence and comparison to homologous

gene products of well studied systems such as *Saccharomyces cerevisiae* or *Neurospora*. It would also allow the construction of strains with targeted knock-outs of the genes of interest (Fonzi & Irwin, 1993) rather than studying the mutation in a genetic background which has been subjected to several rounds of mutagenic treatment. The original goal of this work was to clone a germ tube gene, but that effort was unsuccessful. The cloning approach is described in Part III of this dissertation. Several candidate genes have been isolated (Birse et al., 1993; Liu et al., 1994; Saporito-Irwin et al., 1995) but the deletion of these genes in *C. albicans* does not affect germ tube formation. Nonetheless, these genes do appear to have roles in morphogenesis and their study will undoubtedly yield valuable information about the biology of *C. albicans*.

Genes with roles in morphological conversion have been identified in two other yeasts, *S. cerevisiae* and *Ustilago maydis*. *S. cerevisiae* forms pseudohyphal filaments in response to nitrogen starvation (Gimeno & Fink, 1994) -- a condition which does not elicit filamentous growth in *C. albicans*. However, a shared kinase pathway may transduce signals that induce filamentous growth in these two organisms. In *S. cerevisiae*, mutation of the *STE12* gene encoding the transcription factor Ste12p blocks filamentous growth. A homologue of Ste12p, Cph1p, is required for *C. albicans* to produce hyphae on solid medium but not in serum (Liu et al., 1994). Ste12p is the target of a kinase(s) in the pheromone-responsive kinase cascade. A homologue of Ste7p, a kinase of the above cascade, designated Fuz7p is required by *U. maydis* for hyphal growth. Expression of *FUZ7* can be triggered in response to the pheromone coded by the *a* locus and other putative signals from the plant host (Banuett, 1991; Banuett & Herskowitz, 1994).

Given the above observations, it is tempting to propose that induction of hyphal growth in *C. albicans* may follow a scheme analogous to that of the pheromone-induced responses by *S. cerevisiae*. Given the diversity of the types of media which can induce

filamentous growth in *C. albicans*, it is conceivable that several receptors exist which, in response to the appropriate cues, trigger a signal transduction cascade which modulates expression of genes that cause budding by squelching them, while activating genes necessary for hyphal growth.

Compared to the sexual cycle of *S. cerevisiae*, parasexual manipulation of *C. albicans* is cumbersome. Furthermore, the diploid nature of the *C. albicans* genome requires that investigators are on the lookout for new recessive traits being brought to homozygosity in the course of a parasexual cycle. Nonetheless, the utility of this system has improved over the past few years. There are now several markers which have functions beyond that of merely being observable. Two markers, *gal1* and *ura3*, can be selected against after a fusion to select for hybrids, and can be selected for after heat shock or UV irradiation to select for segregants. The latter use can easily increase the efficiency of recovering segregants by several hundred fold, since the frequency of segregants among heat shock or UV survivors is about 0.5 to 1%. Another especially useful marker is *MPA1*, which, in an auxotrophic strain, allows parasexual analysis of prototrophic isolates (Goshorn & Scherer, 1989), thus precluding the need for mutagenic treatment. The *sfi86* marker also proved invaluable during this work by providing a way to identify segregants visually and for establishing the identity of the *MPA1/SFI86* linkage group.

PART II  
PARASEXUAL GENETIC ANALYSIS OF GERM  
TUBE DEFECTIVE *CANDIDA ALBICANS* MUTANTS

## CHAPTER I

### INTRODUCTION

*Candida albicans* is an asexual fungus which is commonly found in the gastrointestinal tract of healthy humans (Kreger-van Rij, 1984; Odds, 1988). It is also the predominant cause of recurrent vaginal yeast infections (Odds, 1988). However, when the immune system is compromised by modern therapies such as anti-transplant rejection drugs, aggressive chemotherapy, or in cases of diseases which directly impair immunity, *C. albicans* has the capacity to invade internal organs and cause life threatening disease. Among several characteristics considered to be virulence factors, (Odds, 1994; Gow, 1994) is the ability of the organism to interconvert between a budding yeast form and a branching filamentous form. Although both forms are usually observed in patients, it has been proposed that the apically growing hyphal form enhances the pathogen's ability to cause infection by adhering better to epithelium (Sobel et al., 1984), evading phagocytosis (Reynolds & Braude, 1956; Stanley & Hurley, 1969; Arai et al., 1977), and mechanical penetration of host tissue (Ryley & Ryley, 1990; Gow 1994).

Several mutants have been reported which lack the ability to convert from yeast to hyphae (Buckley et al., 1982; Cannon 1986; Howard et al., 1986; Elorza et al., 1994); however, little is known about the number of different genes these mutations may represent, or the linkage groups to which they may belong. Mapping new genes is in itself of fundamental importance to the characterization of this and other phenotypes in *C. albicans*. The recent increase in reports of cloned genes (Scherer, 1995) in combination with the construction of an *Sfi* I macrorestriction map of the *C. albicans* genome (Chu et al., 1993) will soon provide a valuable reference which will facilitate genetic studies of this organism. In practice, a priority for characterizing a new gene should be to map its

position, since mapping a mutation to a previously characterized locus may provide insight into the gene's function and should help avoid undue duplication of effort. The goals of mapping, identifying, and, ultimately, cloning genes that are essential for the interconversion of *C. albicans* between yeast and hyphae might best be achieved by a combination of two complementary approaches; classical and molecular. Attempts to clone such genes directly by complementing the lost function with a genomic library (Part III of this dissertation), selective cDNA library (Birse et al., 1993; Saporito-Irwin et al., 1995), or by inferring gene function from expression in a *Saccharomyces cerevisiae* system (Gimeno & Fink, 1994) have not yet borne fruit. The difficulties in these approaches arise from the lack of a way to select for the restored function, the necessity for disproportionately high expression of mRNA, and dependence on a system which cannot express the trait of interest. However, once a gene has been cloned, it is usually a simple matter to add it to a physical map of the genome.

The classical approach to mapping a mutation involves mating the new mutant with characterized strains having the same or similar phenotype. Lack of complementation of the phenotype almost always indicates allelism (Sherman & Wakem, 1991). Following the phenotype of interest through meiosis should demonstrate whether the phenotype is the result of a single mutation. Further crosses are performed with well characterized mapping strains in order to determine the new mutations chromosomal assignment. Subsequently, mitotic recombination mapping is used to demonstrate which arm of the chromosome contains the gene. Finally, tetrad analyses of crosses between the new mutant and a tester strain with multiple markers on the appropriate chromosome arm are used to more precisely determine the position of the new allele.

For a sexually reproducing yeast like *S. cerevisiae*, the classical approach is straightforward. A vast number of research hours has produced a large bank of well-

characterized tester strains, each dense with markers. *C. albicans*, on the other hand, presents several complications, some of which can be surmounted; but the route is anything but straightforward, as *C. albicans* is asexual and diploid (Whelan & Magee, 1981; Riggsby et al., 1982). To approximate the classical genetic analysis of perfect yeasts, a parasexual cycle has been developed over the past twenty years which has become well established. The five steps of the cycle are: *i*) fusion of diploid strains to produce complementing heterokaryons and tetraploids, *ii*) selection of stable hybrids, *iii*) induction of chromosome loss in tetraploids to produce "paradiploid" segregants which have a new combination of parental chromosomes, *iv*) analysis/manipulation of paradiploids by induced mitotic recombination, and *v*) fusion of a diploid strain with a paradiploid or a fusion between paradiploids to repeat the cycle.

To determine whether similar phenotypes in various strains are due to mutations in the same gene, pairs of mutants must be subjected to spheroplast fusion, and the hybrids analyzed for complementation. *Failure* of the parent strains to complement each other for the trait of interest indicates that the same gene is defective in both parents. If the trait is complemented in the hybrid, the parents are likely to have mutations in different genes which give similar phenotypes. The method used to generate and isolate hybrids requires that the parents have suitable markers, such as stable auxotrophies, to allow for selection of hybrids and counter-selection against parents.

In order to isolate the hybrids, parents with complementary genotypes are converted to spheroplasts and combined in the presence of polyethylene glycol and calcium ions. The fusion mixture is plated on medium which will allow only fusants to grow. For example, if the parents are both doubly auxotrophic, the fusants can be selected on minimal medium. The double auxotrophies of the parental strains makes it unlikely that revertants will arise (Sarachek et al., 1982; Poulter & Rikkerink, 1983). Two types of fusant colonies are

observed from a fusion mixture. Fast-growing, stable prototrophs occur at a low frequency (1 to 100 per  $10^8$  pairs) This class of fusant was shown to be uninucleate with a DNA content consistent with tetraploidy, or in one case, hexaploidy (Sarachek et al., 1982, Whelan et al., 1985).

Slow-growing, irregular colonies arise at a frequency of about 1 per  $10^5$  pairs. This class has multi-nucleated cells which segregate parental-type, uninucleate cells. Heterokaryons give rise to hybrids over time (Sarachek et al., 1982).

## CHAPTER II

### MATERIALS AND METHODS

#### ORGANISMS AND MEDIA

##### *Candida albicans* Strains

The characteristics of strains used in this study are summarized in table 1. Strains SR1181, SR1186, and SR1187 are double auxotrophs which were provided by Alvin Sarachek, Wichita State University. The quadruply auxotrophic mycophenolic acid-resistant (Mpa<sup>R</sup>) strain SR1059 was provided by Stewart Scherer, University of Minnesota. This strain, originally designated "1006" by Scherer's group, has been used for mapping by several laboratories (Goshorn & Scherer, 1989; Iwaguchi et al., 1990; Wickes et al., 1991; Chu et al., 1992; Scherer, 1995). Strain SR1066 is a triple auxotroph with chromosome 2 marked by His<sup>-</sup> auxotrophy due to disruption of the *HIS3* locus and was provided by B. B. Magee, University of Minnesota. Strain SR1260 is a germ tube-negative mutant provided by Dexter Howard, University of California at Los Angeles.

Strains generated during the course of this work are SR1061, SR1064, 2001F, HSD12, and UVR48. Strain SR1061 is a spontaneous germ tube-negative derivative of SR1186. Strain SR1064 is a spontaneous Mpa<sup>R</sup> derivative of SR1061. Strain 2001F is an "exceptional" Gtf<sup>-</sup> hybrid of SR1061 and SR1059. The epithet "exceptional" in this context indicates that most of the hybrids from a particular cross do not possess the exceptional characteristic, in this case Gtf<sup>-</sup>. HSD12 is a heat shock-induced *ura3* homozygous derivative of 2001F. Strain UVR48 is a UV-induced segregant/recombinant derivative of HSD12. The marked linkage groups for SR1059, SR1061, and 2001F are presented in table 2.

Table 1. *Candida albicans* Strains

| A. Parental Strains                                                                                                                                                                                           |                 |                                                  |                                                                                                                               |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|--------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| Strain                                                                                                                                                                                                        | Synonym         | Genotype                                         | Source                                                                                                                        |
| SR1181                                                                                                                                                                                                        | WD-1-6          | <i>lys sfi gtf mpa1</i>                          | AS                                                                                                                            |
| SR1186                                                                                                                                                                                                        | WB-7-5          | <i>ade86 sfi86 GTF1 mpa1 RDN1-1.1</i>            | AS                                                                                                                            |
| SR1059                                                                                                                                                                                                        | 1006            | <i>arg4 lys2 ser57 ura3 gtf MPA1/mpa1 RDN1-1</i> | SS                                                                                                                            |
| SR1260                                                                                                                                                                                                        | UCLA312         | <i>gtf mpa1</i>                                  | DH                                                                                                                            |
| SR1066                                                                                                                                                                                                        | A50122          | <i>gal1 lys2 trp1 his3::λ</i>                    | BM                                                                                                                            |
| B. Parasexual and Spontaneous Derivative Strains                                                                                                                                                              |                 |                                                  |                                                                                                                               |
| Strain                                                                                                                                                                                                        | Parent(s)       | Method                                           | Genotype                                                                                                                      |
| SR1061                                                                                                                                                                                                        | SR1186          | S                                                | <i>ade86 sfi86 gtf1 mpa1 RDN1-1.1</i>                                                                                         |
| SR1064                                                                                                                                                                                                        | SR1061          | S                                                | <i>ade86 sfi86 gtf1 MPA RDN1-1.1</i>                                                                                          |
| 2001F                                                                                                                                                                                                         | SR1061 × SR1059 | F                                                | <i>ARG4/arg4 LYS1/lys1 SER57/ser57 URA3/lura3 MPA1/mpa1 ADE86/ade86 SFI86/sfi86</i>                                           |
| HSD12                                                                                                                                                                                                         | 2001F           | HS                                               | <i>SFI86/sfi86 gtf1 RDN1-1/RDN1-1.1</i><br><i>ura3 ARG4/arg4 LYS1/lys1 SER57/ser57 MPA1/mpa1 ADE86/ade86 SFI86/sfi86 gtf1</i> |
| UVR48                                                                                                                                                                                                         | HSD12           | UV                                               | <i>ura3 sfi86 MPA1/mpa1 gtf1</i>                                                                                              |
| AS - Alvin Sarachek, Wichita State University<br>SS - Stewart Scherer, University of Minnesota<br>DH - Dexter Howard, University of California at Los Angeles<br>BM - Beatrice Magee, University of Minnesota |                 |                                                  |                                                                                                                               |
| S - spontaneous mutation<br>F - spheroplast fusion<br>HS - heat shock<br>UV - UV irradiation                                                                                                                  |                 |                                                  |                                                                                                                               |

Table 2. Genetic Composition and Linkage of SR1059, SR1061, and 2001F.

| Chromosome | SR1059                       | SR1061                       | 2001F                                                           |
|------------|------------------------------|------------------------------|-----------------------------------------------------------------|
| R          | <i>RDN1-1 / RDN1-1</i>       | <i>RDN1-1.1 / RDN1-1.1</i>   | <i>RDN1-1.1/RDN1-1.1/RDN1-1/RDN1-1</i>                          |
| 1          | <i>ser57/ser57</i>           | <i>SER57/SER57</i>           | <i>ser57/ser57/SER57/SER57</i>                                  |
| 2          | <i>MPA1 SFI86/mpa1 SFI86</i> | <i>mpa1 sfi86/mpa1 sfi86</i> | <i>MPA1 SFI86/mpa1 SFI86/mpa1 sfi86/mpa1 sfi86</i> <sup>†</sup> |
| 3          | <i>ura3/ura3</i>             | <i>URA3/URA3</i>             | <i>ura3/ura3/URA3/URA3</i>                                      |
| 4          | <i>lys1/lys1</i>             | <i>LYS1/lys1</i>             | <i>lys1/lys1/lys1/LYS1</i>                                      |
| 7          | <i>arg4/arg4</i>             | <i>ARG4/ARG4</i>             | <i>arg4/arg4/ARG4/ARG4</i>                                      |
| K*         | <i>GTF1/GTF1</i>             | <i>gtf1/gtf1</i>             | <i>gtf1/gtf1</i>                                                |
| L*         |                              |                              |                                                                 |

\* Chromosomes 5 and 6 cannot be genetically identified; the designations K and L have been substituted to accommodate this ambiguity.

<sup>†</sup> Assignment to chromosome 2 is inferred from a cross between strains UVR48 and SR1066.

### **Growth Conditions**

Unless indicated otherwise, all cultures were incubated at 30°C. Cultures for spheroplast preparation were grown to logarithmic phase (approximately  $10^7$  cells/ml) in yeast extract, peptone, and dextrose broth (YPD) as described by Sherman et al. (1986). Routine growth of stocks was on YPD plates or slants containing agar 15 g per liter. Selective growth was conducted on minimal synthetic dextrose medium (SD) described by Sherman et al. (1986) with appropriate supplemental amino and nucleic acids added to 100 µg/ml. Uridine was used to supplement Ura<sup>-</sup> auxotrophs because *C. albicans* Ura<sup>-</sup> auxotrophs cannot be supplemented by uracil (Kelly et al., 1987).

### **Selection with Mycophenolic Acid**

Mycophenolic acid (MPA) was prepared as a 10 mg/ml stock in ethanol and was added to autoclaved media tempered to 50°C to a final concentration of 10 µg/ml. MPA is light sensitive; therefore, media with this antibiotic were stored and incubated in the dark. MPA resistant strains (Mpa<sup>R</sup>) grow better in the absence of the antibiotic, i.e. even resistant strains are inhibited.

### **Germ Tube Test and Hyphal Growth**

Testing for formation of hyphae on solid medium used corn meal agar (Difco) plus 1% Tween 80 (Sigma) at 30° and 37°C (McGinnis, 1980). Induction of germ tube formation in liquid media was carried out both in starch, salts, and vitamins (SSV) medium (Marriott, 1975), supplemented for auxotrophies, and in pooled bovine serum (Taschdjian et al., 1960; Ogletree et al., 1978). Germ tube induction in liquid media was performed at 37°C.

### The Sfi<sup>-</sup> Phenotype

Sulfite reductase pathway mutants (Sfi<sup>-</sup>), are auxotrophic for either cysteine or methionine (Sarachek et al., 1981; Whelan & Soll, 1982) were presumptively identified on bismuth sulfite agar (BiGGY) purchased from Difco. On this medium, Sfi<sup>-</sup> colonies are white, and Sfi<sup>+</sup> are dark brown as shown in figure 1. The Sfi<sup>-</sup> auxotrophy was subsequently verified by testing for growth on SD plates with and without methionine.

### Description of Germ Tube-Defective Mutants SR1181, SR1059 and SR1061

Germ tube mutants (Gtf<sup>-</sup>) were sought among strains which were already multiply auxotrophic, since they could be used in complementation experiments without further treatment. Strain SR1181 (Lys<sup>-</sup> Sfi<sup>-</sup> Gtf<sup>-</sup>) failed to produce germ tubes in SSV but > 95% of cells produced germ tubes in serum. The germ tube-delayed (Gtf<sup>d</sup>) strain SR1059 (Arg<sup>-</sup> Lys<sup>-</sup> Ser<sup>-</sup> Ura<sup>-</sup> Mpa<sup>R</sup> Gtf<sup>d</sup>) produces germ tubes after extended incubation (5 h) in serum or SSV.

Strain SR1061 (Ade<sup>-</sup>, Sfi<sup>-</sup>, Gtf<sup>-</sup>) is a white adenine auxotroph, sulfite reductase-negative, *LYS1/lys1* heterozygote, germ tube-negative variant which was isolated from a germ tube-positive double auxotroph using the following procedure. Strains which were not germ tube-defective (Gtf<sup>+</sup>) were screened for spontaneous production of germ tube mutants according to the procedure of Cannon (1986). *C. albicans* strains 1183, 1184, and SR1186 were subjected to multiple rounds of germ tube induction and hyphal growth followed by filtration to exclude hyphae. Yeast cells that passed through the sintered glass filter were used to inoculate fresh medium, and the cycle was repeated.

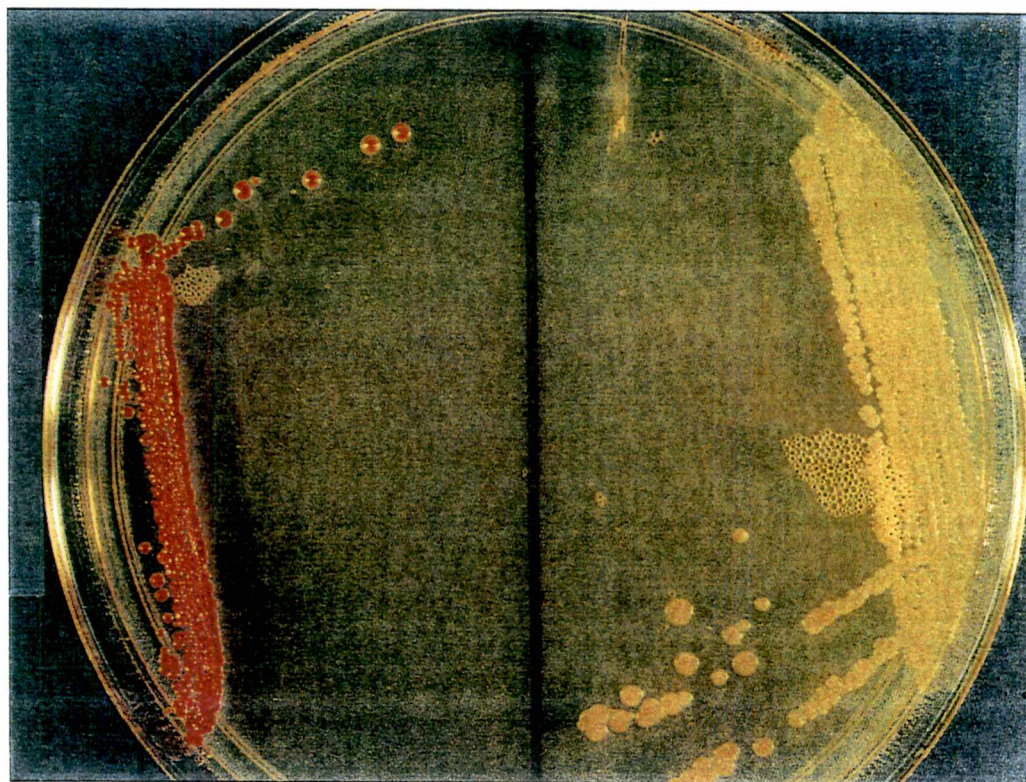


FIGURE 1. Sfi<sup>+</sup> and Sfi<sup>-</sup> colonies on BiGGY agar. *C. albicans* strains 2001F (left) and SR1061 (right) after incubation for 72 h at 30°C on BiGGY agar. Strain 2001F is Sfi<sup>+</sup>; strain SR1061 is Sfi<sup>-</sup>.

Initial cultures were started by inoculating 5 ml of SD broth in 20 mm diameter glass tubes with cells from isolated colonies. Cultures were incubated 24 hours at 30°C with shaking at 200 rpm. Five ml of cell suspension were added to prewarmed 50 ml of SSV broth in 250 ml flasks. The SSV broth was incubated at 37°C without aeration for 5 hours. Samples of germ tube-induced cultures were microscopically examined to monitor germ tube and hyphal growth. After 5 hours, most hyphae were in excess of 50 µm long. The cultures were filtered through sintered glass of porosity C (Pyrex) to exclude hyphae; the filtrate, which was presumed to contain only yeast cells, was filtered through a 20 cm<sup>2</sup> Metrical membrane (Gelman Sciences). The Metrical membrane was transferred to a 20 mm diameter tube containing 5 ml of SD broth supplemented for relevant auxotrophies and incubated overnight with shaking at 30°C. For the next round of induction and filtration, the 5 ml overnight SD cultures were used to inoculate prewarmed 50 ml SSV broth, and the cycle was repeated as above.

Variant SR1061 was isolated as a spontaneous Gtf<sup>-</sup> derivative of SR1186 after four rounds of induction and filtration. The other strains tested failed to yield Gtf<sup>-</sup> mutants after twelve rounds.

#### **Isolation of SR1064, an MPA-Resistant Derivative of SR1061**

Strain SR1061 (Ade<sup>-</sup> Sfi<sup>-</sup> Gtf<sup>-</sup> Mpa<sup>S</sup>), a white adenine auxotroph, was spread at a density of 10<sup>8</sup> cells per plate onto 10 SD plates containing 10 µg/ml MPA. After ten days, five resistant colonies were recovered and designated R1 through R5. One of these, SR1064 (Ade<sup>-</sup> Sfi<sup>-</sup> Gtf<sup>-</sup> Mpa<sup>R</sup>), was used for spheroplast fusion with prototrophic strains.

### **Spheroplast Preparation**

Cultures were grown to log phase in 50 ml YPD in 500 ml flasks with shaking. Cells were harvested in a benchtop centrifuge at 3,000 rpm for 10 min. The cells were resuspended in 10 ml of 1 M sorbitol plus 1%  $\beta$ -mercaptoethanol and incubated for 30 min at 30°C, pelleted at  $1,500 \times g$  for 5 min and resuspended in 5 ml 1 M sorbitol/10 mM Tris•HCl pH 7.4 (ST buffer). Filter sterilized yeast lytic enzyme 70,000 U/g (ICN Biochemicals) was prepared as a stock [10 mg/ml in standard enzyme buffer and stored at -20°C (Sambrook et al., 1989)], and 50  $\mu$ l of stock solution were added to each tube of cells to be spheroplasted. The cells were incubated in the enzyme solution at 30°C with gentle shaking and samples were withdrawn every 15 min and evaluated for conversion to spheroplasts by microscopic examination. Conversion was essentially complete (>95%) after 45 minutes.

Spheroplasts were pelleted by centrifugation at  $1,000 \times g$  for 5 min and washed twice in 5 ml 1M sorbitol/10 mM Tris•HCl (pH 7.4)/10 mM  $\text{CaCl}_2$  (STC). Spheroplasts were resuspended in 5 ml STC, giving approximately  $10^8$  spheroplasts per ml.

## **PARASEXUAL GENETIC TECHNIQUES**

### **Spheroplast Fusion**

For fusions which were not selected on MPA,  $10^8$  of each fusion partner in STC were mixed, pelleted by centrifugation ( $1,000 \times g$  for 5 min) and resuspended in 5 ml of 40% polyethylene glycol (3,700 mw)/10 mM  $\text{CaCl}_2$ . The suspension was then pelleted by centrifugation at  $200 \times g$  for 1 min and incubated for 20 min at 30°C. Following incubation, the mixture was centrifuged at  $1,000 \times g$  for 4 min and decanted. The pellet was resuspended in 1 ml of STC, and 0.1 ml portions were spread onto the surface of selective regeneration agar. Numerous slow-growing colonies (approximately 1,000 per

fusion mixture) of heterokaryons were visible after a week. Infrequent fast-growing hybrid colonies (approximately 10 to 20 per fusion) were visible after 2 to 3 days. Over the course of 7 to 14 days, heterokaryon colonies give rise to fast-growing sectors of hybrids (Sarachek et al., 1981).

### **Selection of Prototrophic Hybrids**

Prototrophic fusants were selected on SD regeneration agar which was osmotically stabilizing (Davis, 1985; Ferenczy, 1985) with either 1 M sorbitol (Hinnen et al., 1978; Kakar & Magee, 1982) or 0.6 M KCl (Sarachek et al., 1982). Hybrid colonies were identified as smooth, fast-growing colonies which were stable for prototrophy after growth in rich medium. Heterokaryons produce irregular colonies which segregate parental nuclei in rich medium. Heterokaryons were not used in any of the experiments in this study.

### **Selection of MPA-Resistant Hybrids**

Fusion mixtures for selection on MPA-containing media contained resistant and sensitive partners in a ratio of 3:1 (Goshorn & Scherer, 1989). Fusants were selected on SD with MPA added after sterilization to 10 µg/ml. The medium was also osmotically stabilizing as described above. Only the fast-growing hybrid class of fusant is recovered after selection on MPA. This result suggests that the allele which confers resistance to MPA is dominant in uninuclear hybrids but recessive in heterokaryons (Goshorn et al., 1989).

### **Hybrid Chromosomal Reassortment and Recombination**

*C. albicans* hybrids typically contain approximately tetraploid amounts of DNA, although there are notable exceptions (Sarachek et al., 1981; Hilton et al., 1985; Whelan et al., 1985). Exceptional hybrids (Goshorn & Scherer, 1989) such as 2001F appear to result

from imperfect karyogamy which results in a genome which is tetrasomic for most chromosomes while remaining disomic for the unselected chromosome.

Two methods were used to derive recombinants from hybrids. Both heat shock and UV irradiation appear to induce chromosome loss in hybrids ( $4n$ ) in the experiments reported here. The use of heat shock to reduce the ploidy of hybrids is well established (Hilton et al., 1985; Goshorn & Scherer, 1989; Chu et al., 1992) however, there is no previous report of UV-induced chromosome loss as described below. Unlike heat shock, UV-irradiation induces mitotic crossing over in addition to causing chromosome loss in tetraploid hybrids. Crossing over brings genes to homzygosity from the crossover point to the end of the arm (Esposito, 1978; Poulter, 1990). UV irradiation induces crossing over in hybrids and paradiploids or natual diploids.

### **Heat Shock-Induced Chromosome Loss**

Fusion products were heat shocked for 90 s at  $51^{\circ}\text{C}$  as described by Hilton et al., (1985). Logarithmically growing cells were added (0.1 ml) to 10 ml of prewarmed YPD broth in a 50 ml flask. After 90 s the flask was chilled in an ice bath. Heat shock survivors were plated on YPD and replica plated on to SD to identify auxotrophs, or were spread onto BiGGY agar and screened for white ( $\text{Sfi}^{-}$ ) colonies.

### **UV-Induced Chromosome Loss**

Fresh overnight cultures of HSD12 and 2001F were appropriately diluted and 0.1 ml portions were spread onto BiGGY plates supplemented with L-arginine, L-lysine, L-serine, L-methionine, adenine, and uridine, and allowed to dry for 30 min. The plates were then irradiated for 10 s by a 15 watt germicidal lamp (General Electric G15T8) suspended 43 cm

above the plates. This dose resulted in approximately 65% killing. White colonies were isolated from the BiGGY plates and tested for other auxotrophies.

### UV-Induced Mitotic Crossing Over

Mitotic crossing over was induced with the same UV dose described above for chromosome loss. Growth conditions for strains HSD12 and 2001F were also the same as described above. Putative recombinants were identified as white (*Sfi*<sup>-</sup>) colonies on BiGGY agar.

Strain SR1061 was tested for heterozygosity at the *LYS1* locus, i.e. *LYS1/lys1*. Such heterozygosity would be expected to yield predominantly Lys<sup>-</sup> auxotrophs among the auxotrophs recovered after UV irradiation as a result of mitotic crossing over. *De novo* mutations to auxotrophy would be less frequent (Whelan & Magee, 1981; Poulter, 1987). Strain SR1061 was spread onto YPD plates at a density of 200 cells per plate and irradiated as described above. After 48 h, surviving colonies were replica plated onto SD plates supplemented with adenine and L-methionine for the existing SR1061 auxotrophies. Replica plates were incubated for 48 h before screening for auxotrophs.

## DNA TECHNIQUES

### Whole Cell DNA Isolation

*Candida albicans* DNA was prepared for restriction endonuclease digests from 10 ml cultures by the glass bead rupturing method of Hoffman & Winston (1987) followed by extraction with phenol, phenol/chloroform/isoamyl alcohol (25:24:1), and chloroform/isoamyl alcohol (24:1).

### **Restriction Endonuclease Digestion and DNA Gel Electrophoresis**

Restriction endonucleases were purchased from Gibco BRL and used according to the manufacturers directions. DNA fragments were separated on agarose gels (Sea Kem® GTG, FMC) according to the parameters specified below. Electrophoresis buffers TAE and TBE have been described (Sambrook et al., 1989).

## CHAPTER III

## RESULTS

**COMPLEMENTATION EXPERIMENTS OF THE GERM TUBE-DEFECTIVE  
MUTANTS**

Six parasexual crosses were performed to test for complementation of the *Gtf*<sup>-</sup> phenotype of four independently derived strains. The germ tube phenotypes of the parents are shown in table 3, and the results of the complementation experiments are summarized in table 4.

**Fusion of SR1181 and SR1061**

Strains SR1181 (*Lys*<sup>-</sup> *Sfi*<sup>-</sup> *Gtf*<sup>-</sup>) and SR1061 (*Ade*<sup>-</sup> *Sfi*<sup>-</sup> *Gtf*<sup>-</sup>) are both doubly auxotrophic germ tube-negative variants. Earlier work has shown that the *Sfi*<sup>-</sup> auxotrophies of the two strains are nonallelic (Sarachek et al., 1982). Spheroplasts of the two strains were fused and hybrids were selected on osmotically stabilized SD plates. Thirty-three prototrophic hybrids were isolated and tested for the ability to produce germ tubes in SSV. Twenty-eight of the hybrids were *Gtf*<sup>+</sup> in SSV, and five were *Gtf*<sup>-</sup>. The five *Gtf*<sup>-</sup> hybrids were tested for germ tube production in serum. Three of the five produced germ tubes in serum, while two failed to produce germ tubes in serum. These results indicate that the germ tube-negative phenotypes of SR1181 and SR1061 are due to recessive mutations in different genes. In the majority of the hybrids (28/33) both *gtf* mutations are complemented, and in 5/33 hybrids, one of the mutations is uncomplemented.

Table 3. Germ Tube Formation of Parental *C. albicans*

| Strain  | Serum | SSV |
|---------|-------|-----|
| SR1186  | +     | +   |
| SR1187  | +     | +   |
| SR1181  | +     | —   |
| SR1059  | d     | d   |
| SR1061  | —     | —   |
| SR1064† | —     | —   |
| SR1260  | —     | —   |
| HSD12   | —     | —   |

The strains were incubated in the designated medium at 37° C and scored after 2 h and 5 h.

d = germ tube formation after 5 h

† SR1064 is a spontaneous Mpa<sup>R</sup> derivative of SR1061

Table 4. Fusion with Germ Tube Mutants

| Hybrids         | Gtf <sup>+</sup> | Gtf <sup>-</sup> |
|-----------------|------------------|------------------|
| SR1181 × SR1061 | 28/33            | 5/33             |
| SR1187 × SR1061 | 23/23            | 0/23             |
| SR1061 × SR1059 | 21/22            | 1/22             |
| SR1181 × SR1059 | 34/36            | 2/36             |
| SR1260 × SR1064 | 0/40             | 40/40            |
| SR1260 × SR1059 | 7/7              | 0/7              |

### **Fusion of SR1187 and SR1061**

Strain SR1061 (Ade<sup>-</sup> Sfi<sup>-</sup> Gtf<sup>-</sup>) was fused with a germ tube-positive strain, SR1187 (Ade<sup>-</sup> Leu<sup>-</sup> Gtf<sup>+</sup>). The adenine auxotrophies of these two strain are nonallelic (Sarachek et al., 1982). From this cross, twenty-three hybrids were isolated and tested in SSV for the ability to produce germ tubes. All twenty-three were found to be Gtf<sup>+</sup>. The result of this cross supports the conclusion that the Gtf<sup>-</sup> phenotype of SR1061 is due to a recessive allele.

### **Fusion of SR1061 and SR1059**

Strain SR1061 (Ade<sup>-</sup> Sfi<sup>-</sup> Gtf<sup>-</sup>) was crossed with the multiply marked strain SR1059 (Arg<sup>-</sup> Lys<sup>-</sup> Ser<sup>-</sup> Ura<sup>-</sup> Mpa<sup>R</sup> *RDNI-1* Gtf<sup>d</sup>). Hybrids were selected on osmotically stabilizing SD medium with MPA. Of the twenty-two hybrids isolated, twenty-one formed germ tubes within 2 h in SSV. One hybrid from this cross produced no germ tubes, even after prolonged (12 h) incubation. The ability of the two germ tube mutants to complement each other indicates that the Gtf<sup>-</sup> phenotype of SR1061 and the Gtf<sup>d</sup> phenotype of SR1059 are due to recessive alleles at different loci.

### **Fusion of SR1181 and SR1059**

Strains SR1181 (Lys<sup>-</sup> Sfi<sup>-</sup> Gtf<sup>-</sup>) and SR1059 (Arg<sup>-</sup> Lys<sup>-</sup> Ser<sup>-</sup> Ura<sup>-</sup> Mpa<sup>R</sup> *RDNI-1* Gtf<sup>d</sup>) were fused and hybrids were selected on osmotically stabilizing SD plates, and thirty-six hybrids were recovered. Of the thirty-six hybrids, thirty-four were able to produce germ tubes within 2 h in SSV. Two of the hybrids were unable to produce germ tubes in SSV, but were able to produce germ tubes in serum within 2 hours. The ability of SR1181

and SR1059 to complement each other's germ tube mutations indicates that the germ tube defects in these strains are the results of mutations at different loci.

### **Fusion of SR1260 and SR1064**

Strain SR1260 is prototrophic and unable to produce germ tubes in either SSV or serum. Because it is prototrophic, it must be fused with a partner which has dominant and recessive selectable markers for which it is complementary. Since the *Gtf*<sup>-</sup> phenotype of SR1260 was most similar to that of SR1061, it was of interest to determine whether they were deficient at the same germ tube locus. There is no way to select for hybrids which would result from a cross between SR1260 and SR1061; therefore, an *Mpa*<sup>R</sup> derivative of SR1061, designated SR1064 (*Ade*<sup>-</sup> *Sfi*<sup>-</sup> *Mpa*<sup>R</sup> *Gtf*<sup>-</sup>), was generated for the purpose of hybridization with prototrophic *Gtf*<sup>-</sup> strains.

Hybrids were selected from the fusion mixture of SR1260 and SR1064 on osmotically supported minimal medium with MPA. Forty hybrids were isolated and tested for germ tube production in SSV. None of the hybrids was capable of germ tube production. Hybrid status of the isolates was verified by heat shock-induced segregation of *Sfi*<sup>-</sup> derivatives from three arbitrarily chosen isolates.

The failure of SR1260 and SR1064 to complement each other's germ tube-negative phenotypes strongly suggests that their mutations are indeed allelic. An alternative explanation is that the allele which causes the germ tube defect in SR1260 is dominant. This interpretation is excluded by the results in the next section.

### **Fusion of SR1260 and SR1059**

To address the question of whether the mutation of SR1260 (prototrophic *Gtf*<sup>-</sup>) was dominant or recessive, it was fused with the *Mpa*<sup>R</sup>, auxotrophic, *Gtf*<sup>d</sup> strain SR1059, and

hybrids were selected on SD with MPA. Seven hybrids were isolated and tested for the ability produce germ tubes in SSV. All seven were  $Gtf^+$ . This result suggests that the germ tube phenotypes of SR1260 and SR1059 are due to mutations in different genes and that the germ tube mutation of SR1260 is recessive. Had the germ tube mutation of SR1260 been dominant, the majority of hybrids would be expected to be  $Gtf^-$ .

### Summary of Complementation Test Results

The complementation tests are most simply interpreted as the four germ tube mutants belonging to three complementation groups: group 1, SR1181; group 2, SR1059; group 3, SR1061 and SR1260. the members of different groups can complement each other, which suggests that the *gtf* mutations are nonallelic. Members of the same group cannot complement each other, which suggests that their *gtf* mutations are allelic.

### MAPPING THE GERM TUBE MUTATION OF SR1061

Parasexual techniques can be used to reveal the chromosome to which a particular locus belongs. Once the locus, *GTF1* in this case, is mapped to a chromosome, it may be possible to locate the gene by chromosome walking (Ausubel et al., 1995).

The nuclear genome of *C. albicans* is comprised of eight pairs of chromosomes which typically range in size from 3 Mbp to 1 Mbp with the ribosomal DNA-containing chromosome varying in size from 3 Mbp to 2 Mbp among strains (Magee et al., 1987; Wickes et al., 1991; Iwaguchi et al., 1990). Most *Candida albicans* geneticists have adopted the convention of designating the seven stable chromosomes 1 through 7, largest to smallest, and designating the variable rDNA-containing chromosome "R".

The fortuitous isolation of a  $Gtf^-$  hybrid, designated 2001F, from the SR1061  $\times$  SR1059 cross provided two opportunities: (i) construction of a *ura3*  $Gtf^-$  derivative which

could serve a host for a *URA3*-bearing vector in experiments to clone the *GTF1* gene, and (ii) mapping the *GTF1* locus to a specific chromosome by parasexual manipulations. The value of opportunity (i) is presented in Part III of this dissertation. The parasexual mapping rationale, (ii), is as follows. Most hybrids possess the entire set of chromosomes from both parents; however, occasionally a hybrid inherits an incomplete set of chromosomes from one of the parents. In the case of 2001F, the SR1059-homologues that contain *GTF1* are not part of the hybrid's genome. As a result, the *gtf1* mutation is left uncomplemented; hence 2001F is *Gtf*<sup>-</sup>. Since the *GTF1*-bearing homologues are absent in 2001F, the 1059-homologues which *are* present in 2001F can be ruled out as possible *GTF1* sites. Chromosomes R, 1, 2, 3, 4, and 7 from SR1059 can be differentiated from the homologous chromosomes from SR1061, either on the basis of a particular phenotype, e.g. *Ser*<sup>-</sup> or *Mpa*<sup>R</sup>, or as in the case of chromosome R, by RFLP analysis (Wickes et al., 1991; Goshorn et al., 1992). Conversely, if a particular SR1059 homologue can be demonstrated to be *absent*, that homologue will be a candidate site for *GTF1*. Hybrid 2001F is prototrophic, while both parents have auxotrophies; therefore, it is likely that the various auxotrophy loci are heterozygous; for example, the hybrid is *Ser*<sup>+</sup>, but heterozygous for the *SER57* locus (*SER57/ser57*). Although, it is conceivable that the hybrid is homozygous (*SER57/SER57*). If, as the result of induced chromosome loss, *Ser*<sup>-</sup> segregants can be produced, then 2001F must possess the SR1059-homologue with *ser57*. If heterozygosity for a particular marker can be established, then the presence of the marker's SR1059-homologue is established. Evidence for heterozygosity of parental markers based on results from heat shock-induced chromosome loss from 2001F (tables 5 & 6) and its *Ura*<sup>-</sup> derivative, HSD12 (tables 7 & 8) is discussed below. Table 2 shows the markers for the chromosomes of SR1059 and SR1061 and the inferred genotype (based on results shown in tables 5 and 7) of 2001F.

Table 5. Heat Shock-Induced Segregation of 2001F

| Number of Segregants | Markers Segregated                |
|----------------------|-----------------------------------|
| 10                   | Lys <sup>-</sup>                  |
| 4                    | Ura <sup>-</sup>                  |
| 2                    | Ser <sup>-</sup>                  |
| 4                    | Ade <sup>-</sup>                  |
| 2                    | Sfi <sup>-</sup> Mpa <sup>S</sup> |
| 2                    | Lys <sup>-</sup> Ade <sup>-</sup> |

Table 6. Frequency of Parental Markers among Heat Shock Segregants of 2001F

| Marker           | Proportion |
|------------------|------------|
| Lys <sup>-</sup> | 12/24      |
| Ura <sup>-</sup> | 4/24       |
| Ser <sup>-</sup> | 2/24       |
| Ade <sup>-</sup> | 6/24       |
| Sfi <sup>-</sup> | 2/24       |
| Mpa <sup>S</sup> | 2/24       |

Table 7. Sfi<sup>-</sup> Heat Shock Auxotrophic Segregants from HSD12

|       | Number | Segregant Phenotype                                                                  |
|-------|--------|--------------------------------------------------------------------------------------|
|       | 17     | Sfi <sup>-</sup> Mpa <sup>S</sup>                                                    |
|       | 12     | Sfi <sup>-</sup> Lys <sup>-</sup> Mpa <sup>S</sup>                                   |
|       | 4      | Sfi <sup>-</sup> Arg <sup>-</sup> Mpa <sup>S</sup>                                   |
|       | 1      | Sfi <sup>-</sup> Ser <sup>-</sup> Mpa <sup>S</sup>                                   |
|       | 4      | Sfi <sup>-</sup> Lys <sup>-</sup> Ser <sup>-</sup> Mpa <sup>S</sup>                  |
|       | 1      | Sfi <sup>-</sup> Arg <sup>-</sup> Lys <sup>-</sup> Ser <sup>-</sup> Mpa <sup>S</sup> |
|       | 1      | Sfi <sup>-</sup> Lys <sup>-</sup> Ade <sup>-</sup> Mpa <sup>S</sup>                  |
| Total | 40     |                                                                                      |

Table 8. Cosegregation Frequency of Parental Markers with Sfi<sup>-</sup> Heat Shock-Induced Auxotrophs from HSD12

| Proportion | Marker           | Frequency |
|------------|------------------|-----------|
| 5/40       | Arg <sup>-</sup> | 12%       |
| 17/40      | Lys <sup>-</sup> | 42%       |
| 6/40       | Ser <sup>-</sup> | 15%       |
| 1/40       | Ade <sup>-</sup> | 2%        |

### Induced Loss of Chromosome 1 by Heat Shock

The Ser<sup>-</sup> auxotrophy of SR1059 results from the mutation of a gene, *SER57*, on chromosome 1 (Goshorn et al., 1992). If *GTF1* were linked *in cis* to *ser57*, then the hybrid 2001F which is homozygous for *gtf1* would not be heterozygous for *SER57*. Hybrid 2001F was shown to be heterozygous for chromosome 1 by heat shock induced-segregation of Ser<sup>-</sup> auxotrophs. Therefore, *GTF1* is not on chromosome 1.

### Induced Loss of Chromosome 3 by Heat Shock

The Ura<sup>-</sup> auxotrophy of SR1059 is the result of a mutation in *URA3* (Goshorn & Scherer, 1989) which is on chromosome 3. If *GTF1* were linked *in cis* to *ura3*, 2001F would not be heterozygous for *URA3* since it is homozygous for *gtf1*. The segregation of Ura<sup>-</sup> auxotrophs from 2001F indicates that the hybrid is heterozygous for chromosome 3. Since 2001F is heterozygous for *URA3*, *GTF1* is not on chromosome 3.

### Induced Loss of Chromosome 4 by Heat Shock

Strain SR1059 is a *lys1* mutant, and results generated during this work suggest that SR1061 is heterozygous for *LYS1*, i.e. *LYS1/lys1*. The *LYS1* locus has been mapped to chromosome 4 (Goshorn et al., 1992). Fifty percent of the auxotrophs isolated after heat shock of 2001F were Lys<sup>-</sup> auxotrophs. These results are consistent with a genomic constitution of simplex tetrasomy (*LYS1/lys1/lys1/lys1*). If *GTF1* were linked *in cis* to the *lys1* alleles of SR1059, 2001F would have to be diploid since it is homozygous for *gtf1*, and therefore, it would be unable to produce Lys<sup>-</sup> segregants. The high frequency of Lys<sup>-</sup> segregants strongly suggests that both SR1059-homologues of chromosome 4 are present in the hybrid; therefore, it cannot be the site of *GTF1*.

### Induced Loss of Chromosome 7 by Heat Shock

Chromosome 7 of SR1059 is marked by a mutant *arg4* allele, which causes auxotrophy for arginine. No Arg<sup>-</sup> auxotrophs were found among the original twenty-four heat shock induced-auxotrophic derivatives of 2001F. However, HSD12, a heat shock-induced Ura<sup>-</sup> segregant of 2001F was heat shocked to induce chromosomal segregation. Segregants were identified as white (Sfi<sup>-</sup>) colonies on BiGGY agar. Among the forty Sfi<sup>-</sup> segregants were five Arg<sup>-</sup> derivatives (Table 7). The presence of the SR1059 chromosome 7 in the hybrid indicates that *GTF1* is not on this chromosome since HSD12 is homozygous for *gtf1* and heterozygous for chromosome 7.

### Heterozygosity of Chromosome R

The R chromosome of *C. albicans* contains a tandem array of rDNA repeats which displays restriction fragment length polymorphism among strains (Magee et al., 1987). Digestion of whole cell DNA with *Hinf* I yields a striking band of approximately 6 kbp in length (Scherer, 1995a) which is comprised of rDNA. The *Hinf*I rDNA fragment of SR1059 migrates at a slightly slower rate than that of SR1061. A *Hinf*I digest of 2001F DNA produces a doublet with bands which correspond to those of the two parental strains (Figure 2). These results suggest that 2001F possesses the R chromosomes of both SR1059 and SR1061. Since 2001F is homozygous *gtf1*, the normal allele, *GTF1*, cannot be on chromosome R.

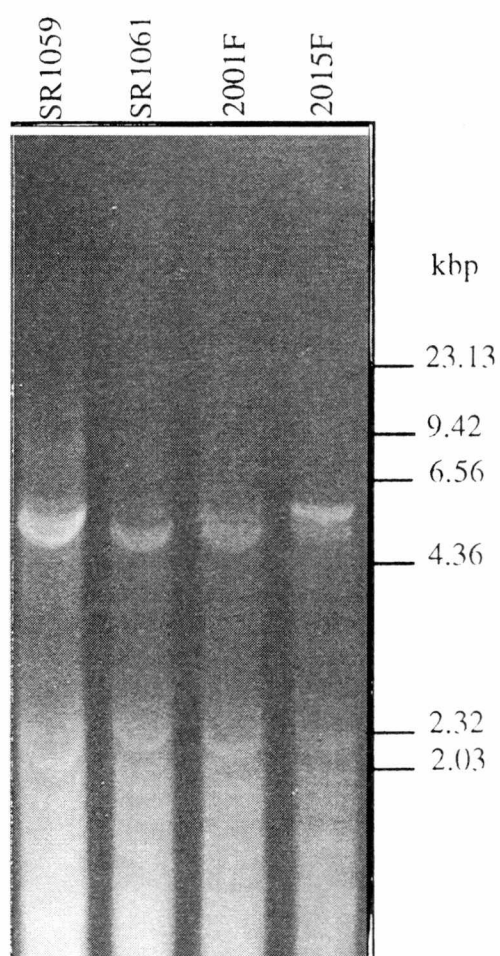


Figure 2. Photograph of EtdBr-stained *Hinf* I-digested whole cell DNA from parental strains SR1059 and SR1061; and hybrid strains 2001F and 2015F. The size markers are *Hind* III fragments of lambda DNA.

### Linkage to Unmapped Markers *MPA1*, *ADE86*, and *SFI86*

Resistance to MPA in SR1059 is conferred by the dominant allele *MPA1*. Goshorn et al., (1989) have shown that *MPA1* is not linked to *RDN* (rDNA); therefore, it is not on chromosome R. Since 2001F is *Mpa*<sup>R</sup>, the chromosome which bears *MPA1* does not contain *GTF1* since 2001F is homozygous for *gtf1*.

The auxotrophies of SR1061, *Sfi*<sup>-</sup> (*sfi86*) and *Ade*<sup>-</sup> (*ade86*) are also unlinked to the germ tube mutation. Since the hybrid 2001F segregates both *Sfi*<sup>-</sup> and *Ade*<sup>-</sup> auxotrophs, it must be heterozygous for both loci. The *sfi86* and *ade86* mutations have not previously been associated with specific chromosomes. Results from this work have provided some insight into the location of these genes. These findings are discussed below.

### Summary of *GTF1* Mapping

The results presented above rule out chromosomes R, 1, 2, 3, 4, and 7 as sites for *GTF1*, leaving only chromosomes 5 and 6 as candidates. The *ADE86* locus is also unlinked to *GTF1*.

### MAPPING *ADE86*

The *ade86* mutation was inherited by 2001F from SR1061; therefore, if it is linked to any of the markers inherited from SR1059 the linkage would be *in trans*. Cosegregation of the *Ade*<sup>-</sup> phenotype with any of the SR1059-inherited markers following heat shock would indicate that *ADE86* is not linked to the cosegregating marker.

The *lys1* and *MPA1* markers were inherited from SR1059 on chromosomes 4 and 2, respectively. If the functional *ADE86* allele were on either of these chromosomes, then

2001F would not produce heat shock-induced segregants which were Ade<sup>-</sup> Lys<sup>-</sup> Mpa<sup>R</sup> since the SR1059-inherited marker would be lost to reveal the SR1061-inherited marker (*ade86*). The hybrid, 2001F, segregated two Ade<sup>-</sup> clones which were also Lys<sup>-</sup> Mpa<sup>R</sup>. Since the presence of SR1059-inherited chromosomes 4 and 2 are required to confer the Lys<sup>-</sup> and Mpa<sup>R</sup> traits, *ADE86* cannot be on chromosomes 4 or 2.

HSD12 was derived from 2001F by heat shock-induced chromosome loss. This procedure rendered the segregant homozygous for *ura3*. The *ura3* allele was inherited from SR1059 on chromosome 3. As argued above, since the *ADE86* alleles of HSD12 were inherited from SR1059, the loss of the SR1059-inherited chromosome which possesses *ADE86* is required to obtain an Ade<sup>-</sup> segregant. If *ADE86* were on chromosome 3, then it would not be possible to generate Ade<sup>-</sup> segregants from HSD12 since it is homozygous for chromosome 3 of SR1059, and hence, homozygous for *ura3*. Since HSD12 produced an Ade<sup>-</sup> segregant after heat shock and two Ade<sup>-</sup> recombinants after UV irradiation, it must still be heterozygous for *ADE86*. Therefore, *ADE86* cannot be on chromosome 3.

Hybrid 2001F is homozygous for the mutant germ tube allele *gtf1*. As argued above, since the Gtf<sup>-</sup> hybrid can segregate Ade<sup>-</sup> derivatives, it must be heterozygous for *ADE86*. Therefore, *ADE86* cannot be on the *GTF1* linkage group, designated linkage group K in table 2.

To summarize, the above results indicate that *ADE86* is not on chromosomes 2, 3, 4, or K. Chromosomes 1, 5, 6, 7, and R remain as possible sites of *ADE86*.

#### DEMONSTRATION OF LINKAGE BETWEEN *SFI86* AND *MPA1*

Linkage was detected between the *MPA1* and *SFI86* genes in chromosome segregation and recombination experiments with the hybrid 2001F and its Ura<sup>-</sup> derivative HSD12. The resistance to MPA is dominant; that is, heterozygotes are resistant. The Sfi<sup>-</sup>

auxotrophy is recessive, which means heterozygotes are prototrophic. Since 2001F inherited the  $Sfi^-$  trait from SR1061 and the  $Mpa^R$  trait from SR1059 the alleles which give these phenotypes, *sfi86* and *MPA1* must reside on different homologues, i.e. *in trans*. Therefore, no segregants derived from 2001F and HSD12 will show both markers,  $Sfi^-$  and  $Mpa^R$ , without crossing over between the loci.

### **Invariant Heat Shock-Induced Cosegregation of $Sfi^-$ and $Mpa^S$ Phenotypes**

Hybrid 2001F was heat shocked and auxotrophic segregants were characterized (table 5) and tested for resistance to MPA. Of the twenty-four auxotrophs characterized, two  $Sfi^-$  isolates were found. These two isolates were also sensitive to MPA ( $Mpa^S$ ). The remaining twenty-two auxotrophs were  $Mpa^R$ .

One of the  $Ura^-$  auxotrophs, HSD12, derived from the above heat shock of 2001F was able to segregate all of the remaining parental markers, which suggests that it is heterozygous for these loci in spite of having been rendered homozygous for the *ura3* marker. Forty  $Sfi^-$  derivatives of HSD12 were isolated after plating heat shocked cells on BiGGY agar. All of these  $Sfi^-$  segregants were found to be  $Mpa^S$ . All of the other parental markers were represented among the  $Sfi^-$  segregants. These results suggest that *MPA1<sup>R</sup>* and *sfi86* are linked *in trans*. Further, these markers represent a linkage group which is separate from the other marked chromosomes in this hybrid as illustrated by the random cosegregation of other parental markers with the *SFI86/MPA1* linkage group. The phenotypes and frequencies of the  $Sfi^-$  HSD12 derivatives are shown in tables 7 and 8.

### **UV-Induced Recombination Between *SFI86* and *MPA1***

Based on the above evidence that *sfi86* and *MPA1* were *in trans*, it was predicted that bringing the *sfi86* allele to homozygosity in HSD12 by UV induced-mitotic crossing over would yield  $Mpa^R$   $Sfi^-$  derivatives which had not been found among the heat shock-

induced derivatives. HSD12 was spread onto BiGGY plates and UV irradiated. Thirty-eight *Sfi*<sup>-</sup> colonies were recovered and tested for resistance to MPA and for parental auxotrophies. One recombinant *Mpa*<sup>R</sup> *Sfi*<sup>-</sup> derivative (UVR48) was found. The remaining thirty-seven *Sfi*<sup>-</sup> derivatives were *Mpa*<sup>S</sup>. As in the above results from heat shock-induced segregation, the other parental auxotrophies occurred randomly among the *Sfi*<sup>-</sup> UV-induced derivatives. Tables 9 and 10 show the phenotypes and frequencies of the UV-induced *Sfi*<sup>-</sup> derivatives. The observed frequency of parental auxotrophies among the UV-induced *Sfi*<sup>-</sup> derivatives was higher than had been expected under the assumption that homozygosity at any of the markers would be the result of mitotic crossing over. A more detailed analysis of the distribution of the *Sfi*<sup>-</sup> heat shock- and UV-induced derivatives is presented below.

### ***MPA1* and *SFI86* Map to Chromosome 2**

To test whether the *MPA1/SFI86* linkage group was in fact chromosome 2, the HSD12-derived recombinant UVR48 (*Mpa*<sup>R</sup> *Sfi*<sup>-</sup> *Ura*<sup>-</sup>) was crossed with strain SR1066 (*Gal*<sup>-</sup> *Trp*<sup>-</sup> *His*<sup>-</sup>) which has a disrupted *HIS3* gene on chromosome 2 (Magee, 1995). Hybrids were selected on osmotically stabilized SD plates which were supplemented with L-histidine in order to allow *His*<sup>-</sup> hybrids to grow. If *MPA1* were on chromosome 2, then all *His*<sup>-</sup> hybrids would be *Mpa*<sup>S</sup>. Since the *Mpa*<sup>R</sup> locus is probably heterozygous [*MPA1/mpa1*] (Goshorn & Scherer, 1989; Scherer, 1995), some of the *His*<sup>+</sup> hybrids would also be *Mpa*<sup>S</sup>, although the majority would be *Mpa*<sup>R</sup>. From the fusion, 125 hybrids were recovered. These hybrids were replicated onto SD, SD with L-histidine, and SD with L-histidine and MPA in order to identify *His*<sup>-</sup>, *His*<sup>+</sup>, and *Mpa*<sup>R</sup> hybrids. Twenty-one *His*<sup>-</sup> *Mpa*<sup>S</sup> hybrids and twenty *His*<sup>+</sup> *Mpa*<sup>S</sup> hybrids were identified. The remaining eighty-four hybrids were all *His*<sup>+</sup> *Mpa*<sup>R</sup>. No *His*<sup>-</sup> *Mpa*<sup>R</sup> hybrids were found. These results are

Table 9. Sfi<sup>-</sup> UV-Induced Auxotrophic Segregants/Recombinants from HSD12

| Number | Segregant Phenotype                                                                                   |
|--------|-------------------------------------------------------------------------------------------------------|
| 15     | Sfi <sup>-</sup> Mpa <sup>S</sup>                                                                     |
| 1      | Sfi <sup>-</sup> Mpa <sup>R</sup>                                                                     |
| 12     | Sfi <sup>-</sup> Lys <sup>-</sup> Mpa <sup>S</sup>                                                    |
| 3      | Sfi <sup>-</sup> Lys <sup>-</sup> Ser <sup>-</sup> Mpa <sup>S</sup>                                   |
| 3      | Sfi <sup>-</sup> Arg <sup>-</sup> Lys <sup>-</sup> Mpa <sup>S</sup>                                   |
| 1      | Sfi <sup>-</sup> Arg <sup>-</sup> Mpa <sup>S</sup>                                                    |
| 1      | Sfi <sup>-</sup> Ser <sup>-</sup> Mpa <sup>S</sup>                                                    |
| 1      | Sfi <sup>-</sup> Ade <sup>-</sup> Arg <sup>-</sup> Lys <sup>-</sup> Ser <sup>-</sup> Mpa <sup>S</sup> |
| 1      | Sfi <sup>-</sup> Ade <sup>-</sup> Mpa <sup>S</sup>                                                    |
| Total  | 38                                                                                                    |

Table 10. Cosegregation Frequency of Parental Markers with UV-Induced Sfi<sup>-</sup>

| Proportion | Marker           | Frequency |
|------------|------------------|-----------|
| 5/38       | Arg <sup>-</sup> | 13%       |
| 19/38      | Lys <sup>-</sup> | 50%       |
| 5/38       | Ser <sup>-</sup> | 13%       |
| 2/38       | Ade <sup>-</sup> | 5%        |

consistent with a heterozygous  $Mpa^R$  locus on chromosome 2. The results of this fusion experiment are summarized in table 11.

Table 11. SR1066  $\times$  UVR48 Hybrids Selected on SD with L-Histidine.

| Number | Phenotype                |
|--------|--------------------------|
| 84     | $Mpa^R$ His <sup>+</sup> |
| 21     | $Mpa^S$ His <sup>-</sup> |
| 20     | $Mpa^S$ His <sup>+</sup> |

### Heat Shock-Induced Loss of Chromosomes in Hybrids

The number of auxotrophs observed for each possible combination was tested by a chi-square goodness of fit test against numbers predicted by assuming duplex tetrasomy, e.g. *ARG4/ARG4/arg4/arg4*, for *ARG4*, *SER57*, and *ADE86*, and simplex tetrasomy for *LYS1*. The chi-square statistic from this test is 14.5 which is less than the critical value of 25 for 95% confidence and 15 degrees of freedom. Thus the model of random chromosome loss to cause diploidization from a generally tetraploid hybrid is supported.

### Evidence of UV-Induced Chromosome Loss in a Hybrid

Irradiation with UV has been used to bring heterozygous loci to homozygosity by mitotic recombination in diploid *C. albicans* isolates. It was expected that UV irradiation of HSD12 would yield the homozygote of interest,  $Sfi^-$ , at a lower but still detectable rate. Thirty-eight  $Sfi^-$  derivatives were recovered from approximately 3,000 irradiated HSD12 colonies (1.3%). The frequency with which  $Sfi^-$  derivatives were generated from HSD12

is in agreement with results reported for UV-induced recombination in diploid strains (Whelan and Soll, 1982; Kakar & Magee, 1982; Poulter, 1990).

As mentioned above, the observed frequency of parental auxotrophies among the UV-induced  $Sfi^-$  derivatives was higher than had been expected under the assumption that homozygosity at any of the markers would be the result of mitotic crossing over. In fact, the frequency of cosegregation of the  $Arg^-$ ,  $Lys^-$ ,  $Ser^-$ , and  $Ade^-$  markers paralleled those of the heat shock-induced derivatives. The same chi-square goodness of fit test was applied to the data, i.e. a model based on diploidization of tetraploid sets by random chromosome loss. The chi-square statistic based on this model was 19 which is smaller than the critical value of 25 for 95% confidence and 15 degrees of freedom. These results indicate that UV irradiation induces chromosome loss as well as mitotic crossing over in strains which have tetraploid chromosome sets.

## CHAPTER IV

### DISCUSSION

The four independently isolated Gtf<sup>-</sup> strains can produce Gtf<sup>+</sup> hybrids. For this reason, the mutations that cause the Gtf<sup>-</sup> traits are considered recessive. Although the mutants analyzed in this study had recessive mutations, the possibility of dominant mutations in the regulation of the yeast to hyphal conversion cannot be ruled out.

The Gtf<sup>-</sup> mutants SR1061 and SR1260 are unable to complement each other; therefore, their mutations are likely to be in the same gene. Both of these strains also fail to produce germ tubes in serum, which supports the argument that the mutations are allelic.

Since germ tube mutants SR1056, SR1181, and SR1061 are capable of complementation, these mutations should be considered nonallelic.

Strains SR1260, SR1061, SR1181, and SR1059 are all considered to be germ tube mutants; however, there are differences among the mutant phenotypes. Such differences might be accounted for by various mutations in the same gene. For example, it is conceivable that the inability of SR1181 to respond to SSV and the inability of SR1061 to respond to serum are due to different mutations of the same receptor gene. Genetic complementation between these mutants however, almost certainly precludes this hypothetical explanation.

Other mutants have been reported which fail to produce hyphae in one usually favorable medium but are induced in other media. For example, strain SGY423 will not produce hyphae in the synthetic amino acid medium of Lee et al. (1975) but will in synthetic tissue culture medium TC199 (Birse et al., 1993). Other strains fail to produce hyphae in proline medium or *N*-acetylglucosamine medium (Corner et al., 1986; Ogletree et al.,

1978). It should be noted that there are no reports of variants which are not induced in serum but are induced in other liquid media.

In fact, the inability of SR1061 to produce hyphae is not absolute. In spite of its inability to produce germ tubes in serum at 37°C, abundant hyphae are produced in corn meal Tween agar at 25°C but not at 37°C. Buckley et al., (1982) have reported a germ tube mutant with the same characteristics. It could be argued that the mutation is temperature sensitive. However, the pathway for yeast to hypha may be complex. Evidence for this view comes from the work of Liu and co-workers (1994) who constructed a *STE12*-homologue null mutant of *C. albicans*. The mutant produces germ tubes at 37°C in serum normally, but it is incapable of producing hyphae on solid medium at 37°C. It would be of interest to test this construct for complementation and to assess its ability to produce hyphae at 25°C.

The germ tube mutant SR1061 was isolated from SR1186 after only four rounds of enrichment. Although no mutagenic procedures were used, the parent strain has sustained at least two mutagenic events to render it a double auxotroph. Several other double auxotrophs were screened with the same procedure for twelve rounds and yielded no mutants. This suggests that SR1186 is heterozygous for the germ tube mutation. The derivative SR1061 was found during this work to be heterozygous for *lysI* auxotrophy; therefore, it is likely that the parent, SR1186, is heterozygous for *lysI* as well. Also, many clinical isolates have been found to be naturally heterozygous for a variety of auxotrophies, most notably *Sfi*<sup>-</sup> auxotrophy for which > 5% of clinical isolates may be heterozygous (Wehlan & Magee, 1981). For this reason, germ tube-negative isolates to be used in a survey to estimate the number of genes involved in the yeast to hypha transition should come from disparate lines.

This work demonstrates that the parasexual cycle also provides the means for strain construction. A strain with an interesting mutation can be fused with a *ura3*-bearing strain, and segregants carrying the new mutation and *ura3* can be isolated. This approach to strain construction has the advantage of avoiding methods that induce new mutations in the strain of interest. This method has the additional benefit of demonstrating whether the mutation of interest is dominant or recessive.

The generation of UVR48 (*ura3 sfi86 gtf1 MPA1*) from HSD12 (*ura3 gtf1 MPA1*), in addition to two *sfi86 MPA1* recombinants from 2001F as the result of mild UV irradiation demonstrates that even markers *in trans* can be brought to *cis* configuration from a hybrid in a single step to yield a recombinant suitable for another round of fusion. This "single step" procedure apparently causes two events: first, a crossover event to bring *MPA1* and *sfi86* together on the same homologue; second, homologues bearing the *SFI86* alleles must be eliminated to give a *Sfi*<sup>-</sup> segregant.

Locating *MPA1* on chromosome 2 may facilitate cloning this gene. In spite of this gene's ability to confer dominant resistance to mycophenolic acid, efforts to clone it from a genomic library have been unsuccessful (Scherer, 1995a). Now the search for this gene can be narrowed to the 2 *Sfi* I macrorestriction fragments that span chromosome 2 (Chu et al., 1993; Scherer, 1995)

By mapping *sfi86* to chromosome 2, this marker can be used to monitor parasexual and crossover events which involve this chromosome. The advantage of the *sfi* marker is that it produces a visually identifiable phenotype. The location of marker *ade86* has been narrowed to chromosomes R, 1, 5, 6, and 7. The question of whether it is on chromosome R can be answered by analysis of *Hinf*I digests of DNA from *Ade*<sup>-</sup> and *Ade*<sup>+</sup> segregants of 2001F to detect heterozygosity for R. Further parasexual analysis will reveal the location

of *ade86*. If this marker is on chromosome 5 or 6, it will be the first genetic marker for one of these chromosomes.

The results from complementation studies presented here indicate that there are at least three genes which have a role in germ tube production. The mutant alleles of these genes were all shown to be recessive.

The location of *GTFI* has been narrowed to chromosome 5 or 6. Chromosome 5 is 1.23 Mbp and can be cut with *Sfi* I to give two fragments which are 750 kbp and 480 kbp in length. Chromosome 6 is 1.09 Mbp and can be cut with *Sfi* I to give two fragments which are 900 kbp and 190 kbp in length. The entire genome is approximately 17 Mbp. (Chu et al., 1993; Scherer, 1995). Future efforts to clone the gene can be focused on this portion of the genome. A parasexually derived strain which is *gtf1 ura3* was constructed during this work and can serve as a host for transformation with *URA3 C. albicans* vectors.

Recent progress in *C. albicans* molecular genetics will certainly contribute to the utility of parasexual analysis. Scores of genes have been recently cloned, mapped, and sequenced (Scherer, 1995). Targeting these genes for disruption (Gorman, 1991; Fonzi & Irwin, 1993) could provide genetic markers for the chromosomes which are still untagged and could more densely mark chromosomes or chromosome regions of interest.

The phenomena associated with the parasexual cycle itself inspire questions about chromosome stability in response to heat shock and UV. It would also be of interest to determine why certain fusion pairs which are productive (yield numerous hybrids) may be drastically less productive when fused with a different strain which is also productive when fused with other strains (Sarachek et al., 1981).

*S. cerevisiae* has served as a model for eukaryote research, and many techniques which were developed during its study have been applicable, often after modification, to *C.*

*albicans*. The recent advances in *C. albicans* research may establish this organism as a model for asexual diploid yeast research. Recent advances in immunosuppression therapy have led to an increase in emerging yeast pathogens (Odds, 1988; Hazen, 1995). There are also several industrially important yeasts which are asexual and diploid (Rachubinski, 1990) which could be manipulated by parasexual methods. Techniques developed for *C. albicans* may be applicable to these organisms.

PART III  
MOLECULAR GENETIC APPROACH TO  
COMPLEMENTING A GERM TUBE-DEFECTIVE  
MUTANT

## CHAPTER I

### INTRODUCTION

Recent application of molecular genetic techniques to the investigation of fungal morphogenesis has identified genes necessary for conversion between different modes of growth in the corn smut *Ustilago maydis* and the yeast *Saccharomyces cerevisiae*. Efforts to find genes dedicated to the yeast to mycelium conversion in *Candida albicans* are underway in several laboratories. An approach to the isolation of a germ tube function gene (*GTF1*) from opportunistic pathogen *C. albicans* is presented here.

The phytopathogenic basidiomycete *U. maydis* has a non-pathogenic yeast-like phase and a pathogenic dikaryotic phase which is filamentous (Fincham et al., 1979). Molecular and genetic analysis of dimorphism of *U. maydis* has resulted in the identification of several loci required for filamentous growth of the dikaryotic, pathogenic phase, e. g. the *FUZ* genes, *b*-locus, and negative regulator *rtf1*, whose expression appears to be governed by mating type pheromones and host signals (Banuett & Herskowitz, 1994; Banuett, 1991).

*Saccharomyces cerevisiae* diploids and *BUD4* mutant haploids are also capable of filamentous growth under certain starvation conditions. The filamentous growth is regulated by several genes with roles in signal transduction (Gimeno & Fink, 1994).

*C. albicans* is capable of growing in two distinct forms. The yeast form is routinely grown in rich or minimal medium at 30°C at near neutral pH. In response to various environmental factors, apical growth, in the form of true hyphae or pseudohyphae, predominates over ovoid, budding yeasts (Berkhout 1923; Odds, 1988). Standard methods for induction of hyphal growth are incubation at 37°C and pH 6.7 in defined amino acids and salts medium (Lee et al., 1975; Buffo et al., 1984) and incubation in serum at 37°C

(McGinnis, 1980; Odds, 1988). Hyphal growth can also be induced on solid medium made from corn meal or rice extract (McGinnis, 1980). This route of hyphal induction might represent a second pathway, since two mutants incapable of hyphal growth in liquid media are capable of hyphal growth on solid media (Buckley et al., 1982) and a mutant capable of hyphal growth in serum but not on solid medium is described by Liu et al. (1994).

The phenomenon of the yeast to mycelium conversion in *C. albicans* has been studied extensively at the nutritional, structural, and physiological level (Odds, 1988; Magee, 1990; Soll, 1990,). Inhibition of chitin synthesis blocks growth of the hyphal tip (Hilenski et al., 1986). The transcription of actin is increased during hyphal growth and the distribution of actin is polarized toward the growing tip, and compartmentalized into discrete "packets" (Anderson & Soll, 1986).

In hopes of gaining an understanding of the mechanisms of dimorphism in *C. albicans*, efforts have been made to identify the genes necessary for the conversion. Achieving this goal could advance the investigation of the role dimorphism has in the ability of *C. albicans* to very successfully evade destruction by host defenses, colonize human oral, gastro-intestinal, and vaginal epithelium, and, in more extreme cases, to invade internal organs. An understanding of the phenomenon at the molecular level would also provide a relatively simple eukaryotic model for experiments in developmental microbiology.

The tools for molecular genetic analysis in this organism are few and relatively new (Kurtz et al., 1987; Goshorn et al., 1992; Cannon et al., 1992; Fonzi & Irwin, 1993). One strategy has been to express *C. albicans* genes in *S. cerevisiae* mutants deficient in filamentous growth and look for restoration of the wild phenotype (Liu et al., 1994). This approach has identified a homologue of *S. cerevisiae* *STE12* designated *CPH1*. *C. albicans* *cph1* mutants fail to produce hyphae on solid media, but produce germ tubes normally in

serum. Another approach, pursued by Fonzi and coworkers, has identified genes which are differentially expressed in yeast and hyphal forms (Saporito-Irwin et al., 1995; Birse et al., 1993). The functions of these genes has been inferred from the resultant phenotypes of their "knock-out" mutants and sequence similarity to *S. cerevisiae* homologues. Though mutant *C. albicans* strains which are incapable of producing germ tubes exist, the gene or genes which are defective in these mutants have not yet been identified (Buckley et al., 1982; Howard et al., 1986; Cannon, 1986; Gow, 1988; Elorza et al., 1994). Mutations in the genes referred to above as preferentially expressed in hyphae do not prevent germ tube production. Though these genes may have a role in germ tube or hyphal development under certain circumstances, the mutants do not possess the Gtf<sup>-</sup> phenotype.

The goal of this work was to isolate a gene or genes which restore to a Gtf<sup>-</sup> mutant the capability of producing germ tubes.

## CHAPTER II

### MATERIALS AND METHODS

#### ENZYMES

Unless otherwise indicated, DNA modifying enzymes were purchased from Gibco BRL;  $\beta$ -glucuronidase (sterile), hen egg white lysozyme, and RNase A were purchased from Sigma Chemicals. All enzymes were used according to the manufacturers directions.

#### *E. COLI* PROCEDURES

##### Strains and Media

*E. coli* strain DH5 $\alpha$  is described in Sambrook et al. (1989). Its relevant characteristics are: *recA1*  $\Delta(lacZYA-argF)_{U169}$  ( $\Phi80lacZ\Delta M15$ ). If it carries a plasmid which expresses a  $\beta$ -galactosidase omega fragment, this strain will produce blue colonies on X-gal media. DH5 $\alpha$  was routinely grown at 37°C on Luria-Bertani medium (LB) made according to Maniatis et al. (1982). Ampicillin (AMP) was added to LB medium at 50  $\mu$ g/ml; X-gal and IPTG were added at 20  $\mu$ g/ml and 0.1 mM, respectively. All solid media contained 1.5% Bacto-agar (Difco).

##### Transformation

*E. coli* DH5 $\alpha$  was transformed with DNA by electroporation using a BioRad Gene Pulser apparatus according to the manufacturer's protocol. Ligation mixtures were diluted 1:5 in sterile deionized water, and 1  $\mu$ l of the dilution was used for each transformation.

Plasmid DNA was isolated from *E. coli* by alkaline lysis for large scale preparations (Ausubel et al., 1995) and by the LiCl method for plasmid mini-preps (Ausubel et al.,

1995). Plasmid DNA from *E. coli* was usually diluted 1:100 in deionized water, and 1  $\mu$ l of the dilution was used for each transformation.

Plasmid DNA from *C. albicans* was prepared by glass bead / vortexing (Hoffman & Winston, 1987) and diluted 1:5 in deionized water, and 1  $\mu$ l of the dilution was used for each transformation.

### Colony Blots

Colony DNA blots were prepared on 8.2 cm diameter nylon supported nitrocellulose filters (Schleicher & Schuell). Filters were marked for alignment to autoradiograms and replica plates then autoclaved to sterilize. The filters were placed face down on LB with AMP plates until wetted, then were turned over and gently pressed to the surface of the plates with a sterile glass rod. A 200  $\mu$ l inoculum of genomic library containing approximately 10,000 cells was spread onto each filter leaving a border of about 2 mm along the edge of each filter. The filters were incubated on the surface of the plates for 2 h at 37°C. Each filter was then lifted from the plates with sterile forceps and placed face down onto a fresh plate, and registration marks corresponding to those on the filters were made on the bottom of the plates to make a replica which could be aligned with subsequent autoradiograms. The filters were then lifted from the replica plates and placed face up onto fresh plates and incubation was continued at 37°C for 6 h. The cells were then lysed with SDS and NaOH, and DNA was fixed to the filter by baking in a vacuum oven for 2 h at 80°C according to method 1 in Sambrook et al. (1989). Colony blots were probed with <sup>32</sup>P-labeled DNA probes as described below for Southern blots.

## **C. ALBICANS PROCEDURES**

### ***C. albicans* Strains and Culture**

*Candida albicans* strains HSD2 (*ura3*) and HSD12 (*ura3*) were isolated as heat shock-induced *ura3* segregants of the Gtf<sup>-</sup> hybrid 2001F; strain HSD12.2 (*ura3*, *lys1*, *sfi86*, *ade86* *MPA1*) was derived by heat shock treatment of HSD12 (figure 1). Strain SR1040 is a wild type synonymous with the type culture ATCC18804. Yeast extract, peptone, and dextrose medium (YPD) and yeast nitrogen base medium (SD) have been described (Sherman et al., 1986). Amino acids, uridine, and adenine were added when appropriate at 100 µg/ml. *C. albicans* cultures were grown on YPD or supplemented SD at 30°C. Cultures of *C. albicans* for transformation were grown in YPD plus 40 µg/ml adenine (YPAD) as described in Guthrie & Fink, (1991).

### **DNA Isolation**

Whole cell DNA was isolated from early stationary phase cells according to Guthrie & Fink (1991) and was purified by centrifugation in a CsCl<sub>2</sub> gradient (1.7 g/ml) without ethidium bromide at 60,000 rpm in a Ti-80 rotor for 16 h. The DNA formed a visible band which was extracted with a 16 gauge needle and 5 ml syringe. The CsCl<sub>2</sub> was removed by dialysis against 10 mM Tris•HCl–1 mM EDTA, pH 7.5 (TE). The DNA was precipitated with two volumes of ethanol and resuspended in the appropriate buffers for enzymatic treatments. Small scale DNA preparations, i.e. "mini-preps" for transformation of *E. coli* was done according to the glass bead method of Hoffman & Winston (1987). DNA prepared in this manner was diluted 1:5 with deionized water prior to use for *E. coli* electroporation.

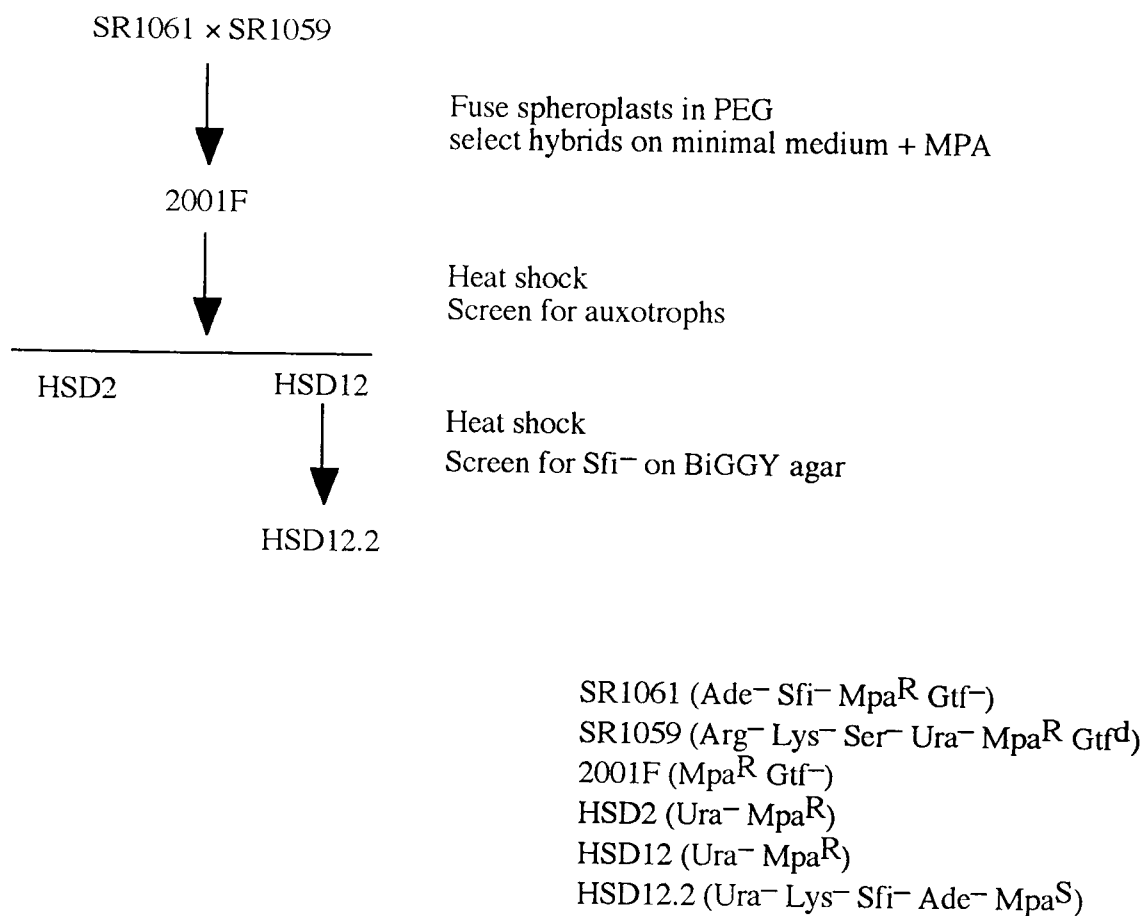


Figure 1. Derivation of strains HSD2, HSD12, HSD12.2 from hybrid 2001F

### Spheroplast Transformation

Spheroplast transformation of *C. albicans* was conducted according to the method of Kurtz et al. (1986) as modified by Cannon et al. (1990) from 50 ml YPAD cultures grown to a density of  $1 - 2 \times 10^7$  cells/ml as determined by a hemocytometer. Typically, 5 - 10  $\mu$ g of plasmid DNA was added to a 200  $\mu$ l volume of spheroplasts prepared according to the above protocol and transformation was effected by the addition of 10 volumes of 20% PEG (3,000 MW). Transformants were selected on appropriately supplemented SD plus 1 M sorbitol (Kurtz et al 1986) and formed visible colonies after 2 to 4 days.

### Germ Tube Screening of *C. albicans* Transformants

When transformed with pRC2312, *ura3 C. albicans* strains produces two types of Ura<sup>+</sup> transformants (Cannon et al., 1990). Transformants which carry free (nonintegrated) plasmid produce slow-growing colonies which segregate Ura<sup>-</sup> cells in the absence of selection, and DNA preparations from these transformants can be used to transform *E. coli*. Transformants with integrated plasmid produce fast-growing colonies which fail to segregate Ura<sup>-</sup> cells when grown under nonselective conditions.

Transformants were screened in pools of approximately 100 transformants per pool; fast-growing transformants with integrated plasmids were pooled separately from slow-growing transformants with unstable plasmids. Pooled cells were incubated overnight in 5 ml of complete minimal broth without uridine (Sherman et al., 1986). Cells were pelleted in a benchtop centrifuge and resuspended in sterile water to give a concentration of approximately  $10^8$  cells/ml. Tubes containing 10 ml of starch, salts, and vitamins (SSV) medium (Cannon, 1986; Marriott, 1975) were warmed to 37°C and 0.1 ml of transformant suspensions were added. The cells were incubated at 37°C for 5 h without aeration.

Suspensions containing HSD2 or HSD12.2 and SR1040 were always included as negative and positive controls, respectively. After 5 h, cells were pelleted and resuspended in 1 ml of sterile water. Samples of each pool were examined by microscopy for the presence of cells with germ tubes. At least 2,000 cells from each pool were examined.

## DNA PROCEDURES

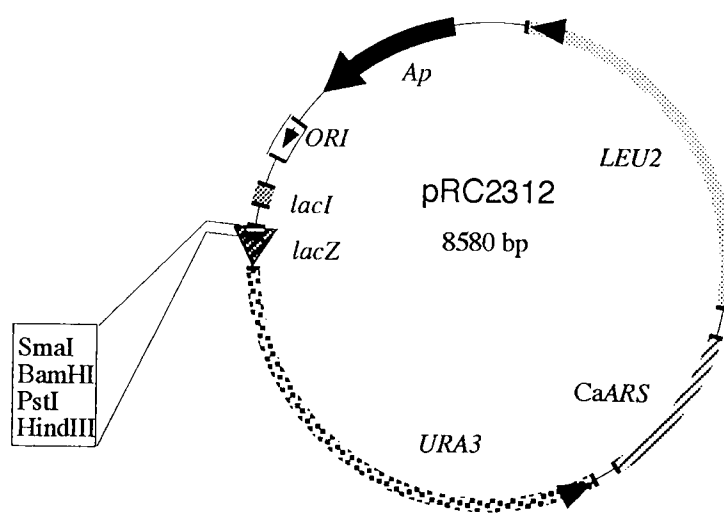
### Agarose Gels and Hybridizations

SeaKem® GTG and SeaPlaque® (low melting-point) agaroses (FMC BioProducts) was made to the concentrations specified for the particular application below. TBE and TAE buffers are described in Sambrook (1989). Nylon membrane (ICN) was used for Southern blots. DNA in gels was stained with ethidium bromide and visualized with long wavelength UV. For facilitating transfer, DNA was nicked with short wavelength UV (Maniatis 1982) and transferred to membranes by vacuum at 20 cm H<sub>2</sub>O for 40 min. using 0.4 M NaOH as the transfer buffer. Prehybridizations for 1 h and hybridizations for 16 h were carried out in APH solution 1 ml per 10 cm<sup>2</sup> [5 × SSC, 1% (w/v) sodium dodecyl sulfate (SDS), 5 × Denhardt Solution (Maniatis, 1982), and 100 µg/ml denatured salmon sperm DNA] at 65°C. One ml of solution was used for each 10 cm<sup>2</sup> of membrane. DNA probes were labeled by [ $\alpha$ -<sup>32</sup>P]dCTP by random primer extension using a Promega Prime-a-Gene® kit to a specific activity of 10<sup>9</sup> - 10<sup>10</sup> dpm/µg. Filters were washed for 2 h at 65°C in 0.1 × SSC, (1 × SSC: 0.15 M NaCl, 15 mM Na<sub>3</sub> citrate), 0.5% (w/v) SDS. Autoradiography was carried out with Kodak XRP film and Cronex® Lightning Plus intensifying screens (Dupont) at -70°C for 12 to 48 h.

### Shuttle Vector

The shuttle vector pRC2312 (Cannon et al., 1992) [figure 2] contains the ampicillin resistance gene (*bla*), replication origin (*ori*), and *lacZ*/multiple cloning site from pUC9 (Ausubel et al., 1995) which permit ampicillin selection, replication in *E. coli*, and blue/white screening, respectively (Sambrook et al., 1989). During this work, it was determined that the *Bam*H I site in the multiple cloning site is unique; this discovery permitted the use of *Sau*3A I for partial digest of genomic insert DNA to give compatible ends for ligation. The vector also contains the following *C. albicans* sequences: *LEU2*, *URA3*, and *CaARS*. The *CaARS* element permits autonomous replication in *C. albicans* and *Saccharomyces cerevisiae*. The *URA3* and *LEU2* genes function in both yeasts as well.

Shuttle vector pRC2312 was digested with *Bam*H I and was treated with calf intestinal alkaline phosphatase. Dephosphorylated vector was purified on 0.7% low-melting-temperature agarose. The vector was freed from the agarose by treatment with agarase (New England Biolabs) followed by ethanol precipitation as described above. Vector and insert DNA were combined in various ratios (from 1:4 to 4:1) and incubated with T4 DNA ligase in a 20  $\mu$ l reaction volume overnight at 4°C. Ligation reactions were diluted five-fold in deionized water, and 1  $\mu$ l samples were electroporated into *E. coli* DH5 $\alpha$ . Transformants were selected on LB plates containing ampicillin, X-gal, and IPTG. Vectors containing inserts produced white colonies, those without inserts produced blue colonies. The ligation reaction containing 2:1 vector to insert ratio produced the highest frequency of vectors with inserts at 86% (198 of 230). DNA mini-preps (Ausubel et al., 1995) from ten white colonies were digested with *Hind* III and electrophoresed (0.6% agarose, 1 $\times$ TAE, 5 V/cm, 3 h); all contained an insert.



 *Candida albicans* Autonomous Replicating Sequence

 *C. albicans* LEU2 gene

 *C. albicans* URA3 gene

The restriction endonuclease site shown are in the multiple cloning site are unique.

Figure 2. *Candida albicans* / *Saccharomyces crervisiae* / *Escherichia coli* shuttle vector pRC2312.

The remainder of this ligation mixture was electroporated into DH5 $\alpha$  as described above and cells were plated on LB with ampicillin to give approximately 400 colonies per plate on 116 plates. After incubation for 2 days, the plates were chilled to 4°C and cells were washed from the plates with LB broth with ampicillin, and divided into three pools containing 23,000; 11,000; and 6,000 clones, designated as libraries 1040-3, 1040-2, and 1040-1, respectively, and incubated for 1 h. The cells were harvested in a GSA rotor at 6,000 rpm for 10 min. The pellets were resuspended in 15 % glycerol and frozen in aliquots.

### Construction of Genomic Libraries

Insert DNA from *C. albicans* strain SR1040 was partially digested with *Sau3AI* (Sambrook et al., 1989). Fragments 7 - 10 kb were separated by electrophoresis in 0.7% agarose – 1 $\times$  TAE at 0.67 volts/cm for 17 h. The fragments were recovered from the agarose gel slice by electroelution in 1 $\times$  TAE at 0.67 volts/cm for 24 h. The eluate was made 150 mM NaCl and extracted once with phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/ isoamyl alcohol (24:1). The DNA was precipitated with 2 volumes of ethanol and resuspended in sterile, deionized water for ligation procedures.

### *Candida albicans* DNA Probes

The *C. albicans* PTR2 15.19 probe was provided by Mark Lubkowitz and consists of a 976 bp portion of that gene, beginning 402 bp downstream of the start codon (Basrai et al., 1995). *C. albicans* mitochondrial DNA was provided by Richard Hurt. The mtDNA was digested with *EcoRI* prior to random primer labeling.

## CHAPTER III

### RESULTS

#### TRANSFORMATION EFFICIENCY

Transformation of *C. albicans* strains HSD2 (Ura<sup>-</sup> Gtf<sup>-</sup>) and HSD12.2 (Ura<sup>-</sup> Lys<sup>-</sup> Ade<sup>-</sup> Sfi<sup>-</sup> Gtf<sup>-</sup>) with the shuttle vector pRC2312 gave Ura<sup>+</sup> colonies at a relatively high frequency. Small colonies appeared on selective medium 2 days after transformation. Transformation efficiencies of HSD2 and HSD12.2 were  $2.1 \times 10^3$  and  $1.3 \times 10^4$  transformants per  $\mu\text{g}$  of pRC2312 DNA respectively.

Transformation of strains HSD2 and HSD12 with genomic library 1040-3 was drastically less efficient than transformation with the unaltered vector. HSD2 and HSD12 were transformed to Ura<sup>+</sup> with an efficiency of 47 and 55 transformants per microgram of 1040-3 DNA.

#### COLONY BLOTTING AND HYBRIDIZATION OF *C. ALBICANS* GENOMIC LIBRARY 1040-3

To determine whether *C. albicans* genes could be recovered from the library, colony DNA blots were prepared, and the filters were probed to estimate the representation of particular sequences in the library.

##### Hybridization with mtDNA

Three filters were probed with <sup>32</sup>P-labeled *C. albicans* mitochondrial DNA (mtDNA). Autoradiography revealed approximately 210 mtDNA-bearing clones per filter or 2.1%.

### Hybridization with *PTR2* probe

To determine whether a single-copy gene could be recovered from the library, five filters were probed with a  $^{32}\text{P}$ -labeled portion of the *C. albicans* *PTR2* gene. Autoradiography revealed from 4 to 10 positive clones per filter (0.04 - 0.1%). Colonies corresponding to the positions of positive signals were subcultured from replica plates for two positive signals from different plates. Cloning of *PTR2* was verified by digestion of plasmid with *Sau3AI* followed by Southern blotting and hybridization with the *PTR2* probe (figure 3).

### ATTEMPTS TO ISOLATE THE *GTF1* GENE FROM LIBRARY 1040-3

*C. albicans* strain HSD2 was transformed with 1040-3 DNA as described above and 2,000 Ura<sup>+</sup> transformants were recovered. The transformants consisted primarily of small slow-growing colonies (95%) and a minority of fast-growing colonies. These two types, small and large, appear to be transformants with free and integrated plasmids. This assumption is supported by the ability of mini-prep DNA from small colonies to transform *E. coli* DH5 $\alpha$  and the failure of large colony DNA to do so.

The transformants were arranged in pools of 100, with the large-colony transformants in a separate pool, and tested as described above for the ability to produce germ tubes. Positive control tests employed strain SR1040 and mixtures of SR1040 and HSD2 in ratios of 1:100 and 1:1000. Cells with germ tubes were not observed in any of the transformant pools, but germ tubes were detected in all of the positive controls.

Transformants of strain HSD12.2, (2,500) were tested in the same manner, but again no germ tube-positive transformants were detected.

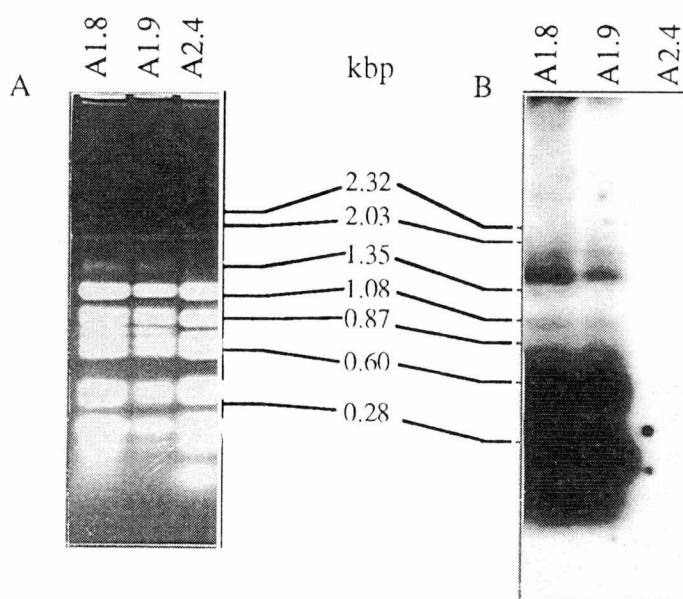


Figure 3. *PTR2* cloned from *C. albicans* library 1040-3. Panel (A) Photograph of EtdBr stained *Sau3A* I-digested plasmid DNA from *PTR2* clones A1.8 and A1.9, and undefined clone A2.4 from *C. albicans* genomic library 1040-3.

Panel (B) Autoradiograph of a Southern blot of the gel in (A) probed with *PTR2* fragment 15.19.

Size markers are *Hind* III fragments of lambda DNA and *Hae* III fragments of  $\Phi$ X DNA.

### ATTEMPTS TO CLONE *ADE86* AND *SFI86*

Samples from the HSD12.2 (*ura3*, *lys1*, *ade86*, *sfi86*)[1040-3] transformant pools diluted to  $5 \times 10^3$  cells/ml and 0.1 ml portions were spread onto supplemented SD minus uridine and adenine and onto supplemented SD minus uridine and methionine. After 3 days, Ade<sup>+</sup>, Ura<sup>+</sup> colonies were found in 2 of the pools, and Sfi<sup>+</sup>, Ura<sup>+</sup> colonies were found in 3 of the pools. These colonies appeared to be fast-growing compared to most HSD12 transformants. The Ura<sup>+</sup>, Ade<sup>+</sup>, and Sfi<sup>+</sup> phenotypes were stable, i.e. they were not segregated after passage on rich medium. Furthermore, DNA mini-preps from these transformants were not able to transform *E. coli* DH5 $\alpha$  to Amp<sup>R</sup>. Thus, it is likely that the vectors carrying the *ADE86* and *SFI86* genes had integrated into the host genome (Goshorn et al., 1992). Another possible, but less likely explanation is that the colonies arose from transformants which reverted simultaneously for Ura<sup>-</sup> and Ade<sup>-</sup>; and Ura<sup>-</sup> and Sfi<sup>-</sup>. The *ura3* allele of HSD12.2 reverts at a frequency of  $10^{-8}$ , and *ade86*, and *sfi86* alleles revert at a frequency of approximately  $10^{-7}$  (Sarachek, 1981).

## CHAPTER IV

## DISCUSSION

Although *C. albicans* has been the object of scientific investigation for many years, its diploid genome and absence of a sexual cycle have precluded the classical strain manipulation techniques which have been exploited in *S. cerevisiae* to genetically characterize interesting strains (Guthrie & Fink, 1991). Hence, there is always uncertainty about whether a strain of interest carries a recessive allele which will at some time be revealed as the result of spontaneous mitotic recombination. Putting these concerns in perspective, several genes have been cloned from the organism, and their functions have been determined by expression in *C. albicans* hosts. However, the function of most *C. albicans* genes have been characterized in well defined *S. cerevisiae* hosts.

Transformation of the paradiploid strains HSD2 and HSD12.2 with the vector pRC2312 gives efficiencies of  $2.1 \times 10^3$  and  $1.3 \times 10^4$  per  $\mu\text{g}$ , a six-fold difference. This is interesting since the two strains share the same parents and, therefore, the same *ura3* mutation which is the basis of selection for transformants. The *ura3* parent gave on the order of  $10^2$  transformants per  $\mu\text{g}$  of pRC2312. These results demonstrate that novel, desirable characteristics can be obtained in the products of parasexual matings of *C. albicans*. The stark difference in transformation efficiencies with the native vector and the genomic library cannot be accounted for by the difference in the number of plasmid molecules per  $\mu\text{g}$  of DNA since there is only a two-fold difference, on average, in size (the vector is approximately 8.6 kb and the insert DNA has an average size of 8.5 kb); the difference in efficiencies is roughly 50 to 200 - fold.

While disappointing, the failure to clone *GTF1* is not unprecedented. Efforts in other laboratories to clone a gene which can restore the ability to form germ tubes to *Gtf*<sup>-</sup> mutants

of *C. albicans* have also been unsuccessful (Birse et al., 1993; Liu et al., 1994; Saporito-Irwin et al., 1995).

Although the effort to clone *GTF1* was unsuccessful, library 1040-3 did contain another single copy gene, *PTR2*. Therefore, this library may be useful for isolating other *C. albicans* genes by expression in either *S. cerevisiae* or *C. albicans*. There is currently only one other *C. albicans* genomic library in a vector which replicates in *E. coli*, *S. cerevisiae*, and *C. albicans*.

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## VITA

On the *very last day* of 1961, Ronald Arthur Uphoff entered the world in Omaha, Nebraska. Despite the intensive efforts of the good Sisters at St. Clement's Elementary School and Cardinal Gibbon's High to speed him up, he has continued to wait until the last minute for almost everything.

The author has taken a postdoctoral position in Dabney Johnson's lab at ORNL where he will study mutant mice. He currently resides in Knoxville, Tennessee with his wife, Jennie and his children, Miranda and Jacob.