YEAST VOL. **13:** 1383–1389 (1997)

Yeast Sequencing Reports

Isolation and Sequence of the Gene Encoding Ornithine Decarboxylase, *SPE1*, from *Candida albicans* by Complementation of a *spe1*Δ Strain of *Saccharomyces cerevisiae*

MARK D. McNEMAR[1], JESSICA A. GORMAN[2] AND HELEN R. BUCKLEY[1]*

[1]*Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, U.S.A.*
[2]*Department of Microbial Genetics, Bristol-Myers Squibb, Princeton, New Jersey, U.S.A.*

Received 5 March 1997; accepted 15 May 1997

The gene encoding ornithine decarboxylase, *SPE1*, from the pathogenic yeast *Candida albicans* has been isolated by complementation of an ornithine decarboxylase-negative (*spe1*Δ) strain of *Saccharomyces cerevisiae*. Four transformants, three of which contain plasmids with the *SPE1* gene, were isolated by selection on polyamine-free medium. The *C. albicans* ornithine decarboxylase (ODC) showed high homology with other eukaryotic ODCs at both the amino acid and nucleic acid levels. The GenBank accession number for this gene is U85005. © 1997 John Wiley & Sons, Ltd.

Yeast 13: 1383–1389, 1997.
No. of Figures: 3. No. of Tables: 0. No. of References: 19.

KEY WORDS — ornithine decarboxylase; *SPE1* gene; *Candida albicans*

INTRODUCTION

Candida albicans is an opportunistic pathogenic fungus that normally exists as a harmless commensal of the gastrointestinal tract of humans and other mammals. In recent years, this fungus has become a source of concern in the clinical setting, causing a wide variety of symptoms ranging from mild to fatal in persons with AIDS, transplant recipients, diabetics, pregnant women, and patients on long-term antibiotic therapy (Odds, 1988). *C. albicans* has a multitude of potential virulence factors, including hypha formation, proteinase production, receptors and adhesins, molecular mimicry of host components, surface hydrophobicity, and contact sensing (Cutler, 1991; Odds, 1994; Sherwood *et al.*, 1992). Of these virulence factors, the ability to switch from yeast to hypha is thought to be necessary for epithelial and deep tissue penetration.

Much study has gone into determining the mechanism(s) by which *C. albicans* alters cell morphology. However, genetic study of this process has been greatly impeded by the lack of a recognized sexual cycle, the diploid nature of the genome, and a paucity of molecular biological tools specifically adapted for use in this species. Many advances in understanding the biology of the fungus have come from drawing parallels

*Correspondence to: Helen R. Buckley, Department of Microbiology and Immunology, Temple University School of Medicine, 3400 N. Broad Street, Philadelphia, PA 19140, U.S.A.

CCC 0749–503X/97/141383–07 $17.50
© 1997 John Wiley & Sons, Ltd.

with other fungi, especially *Saccharomyces cerevisiae*. One enzyme that has been closely linked to differentiation in the fungi is ornithine decarboxylase (ODC). ODC is the first enzyme in the polyamine biosynthetic pathway and its activity is highly regulated during the initial stages of differentiation in many fungi, including *S. cerevisiae* (Tabor *et al.*, 1982), *Neurospora crassa* (Davis, 1990), and *Mucor racemosus* (Inderlied *et al.*, 1980). The competitive inhibitor of ODC, 1,4-diaminobutanone, has been shown to abolish the ability of cultures of *C. albicans* to grow as hyphae, but not their ability to grow normally as yeasts (Martinez *et al.*, 1990).

Because this evidence implicates ODC as a potential role-player in hypha formation, we sought to examine this enzyme in *C. albicans*. As a first step to define a potential role for ODC in morphological transition in *C. albicans*, we have cloned and sequenced the gene encoding ornithine decarboxylase, *SPE1*, from *C. albicans* by complementation of a *spe1*Δ strain of *S. cerevisiae*.

MATERIALS AND METHODS

Culture media and strains

SAB medium was used for stock cultures. A polyamine-free medium (H-PU) was used for isolation of *S. cerevisiae* transformants (Balasundaram *et al.*, 1994). H-PU medium was supplemented with uracil (0·05%) or putrescine (0·1 μg/ml) for selection of cured transformants or maintenance of Y359::pSS1041. YEPD (2% yeast extract, 1% peptone, 2% glucose) was used for curing plasmids from transformants. YNBD (0·67% yeast nitrogen base without amino acids, 2% glucose) alone or supplemented with histidine (0·05%) was used for selection of transformed *S. cerevisiae* strains cured of their transforming plasmids. When solid media were necessary, 2% agar was added. LB (1% tryptone, 0·5% yeast extract, 0·5% NaCl) was used for bacterial cultures. Ampicillin (50 μg/ml), chloramphenicol (35 μg/ml), or tetracycline (50 μg/ml) was added to LB as appropriate for *Escherichia coli* transformant selection. When LB plates were necessary, 1·5% agar was added. *S. cerevisiae* strain Y359 (MATα, *ura3*, *his6*, *leu2*, Δ*spe1::LEU2*) was kindly provided by Dr David Balasundaram (Balasundaram *et al.*, 1994). Bacterial strains used include *E. coli* INV-α (Invitrogen) and DH5-α (Gibco).

Library and plasmids

The *C. albicans* genomic library in the *S. cerevisiae*/*C. albicans*/*E. coli* shuttle vector pSS1041 was provided by Dr Stuart Scherer (Goshorn *et al.*, 1992). The selectable markers of relevance are the *C. albicans URA3* gene and an ampicillin resistance gene. The low-copy number plasmid pACYC184 (New England Biolabs) was used for subcloning and sequencing pY359-2. All manipulations of DNA (isolation, preparation, digestion, and ligation) were performed using standard protocols. Transformation of bacteria or yeast was performed by standard electroporation protocols (Ausubel *et al.*, 1993; Kaiser *et al.*, 1994). All restriction enzymes were purchased from Promega.

Selection of the Δspe1*-complemented transformants*

The ODC-negative (Δ*spe1*) strain of *S. cerevisiae*, Y359, was transformed with a *C. albicans* genomic library contained in the pSS1041 vector (Goshorn *et al.*, 1992). After electroporation, transformants were streaked on H-PU agar and incubated at 37°C until colonies appeared. Each plate containing transformants was overlaid with sterile water (polyamine-free) and the transformants were suspended by gently scraping the agar surface with a sterile loop. The suspension was collected and 100 μl from each plate was transferred to a separate, fresh H-PU plate, streaked for isolation and incubated at 37°C. All colonies from these secondary plates were pooled and streaked for isolation on fresh H-PU plates and incubated at 37°C. Eight individual colonies from these tertiary plates were isolated; however, for analysis of the transforming plasmids, only one colony from each strain was used. All strains were maintained on H-PU plates.

Sequencing, homology search and predicted protein comparison

All sequencing was performed at the DNA sequencing facility, Department of Genetics Core Facility, University of Pennsylvania. The compiled sequence of the *C. albicans SPE1* gene was used to conduct a FASTA search on the GCG database. Translation to the predicted amino acid sequence and alignment of the amino acid sequences from the various ODC proteins was performed using the GCG translation and PILEUP programs, respectively.

 © 1997 John Wiley & Sons, Ltd.

Southern blot analysis of plasmid DNA and C. albicans *genomic DNA*

Plasmid DNA for Southern blot analysis was isolated by standard protocols from bacterial strains to prevent contamination with *S. cerevisiae* genomic DNA. The DNA was digested with *Bam*HI, a single cutter in all plasmids before standard Southern blotting protocols for transfer, hybridization, and probing were followed (Sambrook *et al.*, 1989). Genomic DNA was isolated from three *C. albicans* strains—B311 (ATCC 32354), V6 (Buckley *et al.*, 1982) and V6R (Buckley *et al.*, 1986)—by standard protocols (Kaiser *et al.*, 1994). After digestion with the restriction enzymes mentioned in the text, standard Southern blot protocols were followed to analyse the *SPE1* gene. A 1·1 kb *Eco*RI-*Xba*I fragment of the *SPE1* gene isolated from the pY359-2 plasmid was used as the probe for all Southern analyses. All hybridizations were performed at a temperature of 65°C.

RESULTS AND DISCUSSION

Isolation of the SPE1 *gene from* C. albicans

To determine if it would be possible to isolate the gene encoding ODC from *C. albicans* by homology we performed a Southern blot with a polymerase chain reaction (PCR) product from *S. cerevisiae* obtained using *SPE1*-specific primers. Under all conditions used, no cross-hybridizing bands were detected. Our second approach was to use degenerate PCR primers based on conserved regions from two fungal ODC-encoding genes (Fonzi and Sypherd, 1987; Williams *et al.*, 1992). DNA sequence analysis showed that none of the amplified fragments of the expected size contained an ODC homologous sequence. Therefore, we attempted to isolate the gene by complementation.

Mutants of *S. cerevisiae* that are defective in the gene encoding ODC (*SPE1*) are unable to grow in the absence of added polyamines, because these cells are incapable of making putrescine, spermidine or spermine. However, following a shift from complete medium to a medium lacking polyamines, a long period of growth is necessary to deplete intracellular polyamine stores (Balasundaram *et al.*, 1994). To establish optimal conditions for polyamine depletion, control experiments using a *spe1*Δ mutant, Y359, were performed. The results showed that at least 10 generations of growth in polyamine-free medium were necessary for depletion of the internal polyamine stores and cessation of growth, in agreement with previously determined growth curves for this strain (Balasundaram *et al.*, 1994).

Since initial indications were favorable for use of this strain in a complementation assay, Y359 was transformed with a *C. albicans* library constructed in a yeast shuttle vector. Transformants were plated on defined medium lacking both uracil and polyamines, H-PU medium, to select for total Ura+ transformants and concomitantly deplete these transformants of polyamines. After a 5-day incubation period, the total population growing on each plate was harvested by resuspension in polyamine-free irrigation water and aliquots were plated on fresh H-PU to select for those cells able to grow in the absence of added polyamines. Four independent potential Ura+, *SPE1* colonies were isolated and were termed Y359-2, Y359-6, Y359-9, and Y359-13. Of these four colonies, Y359-2, -9, and -13 grew as well as a wild-type control strain of *S. cerevisiae*. The fourth, Y359-6, grew at a slower rate on H-PU agar and not at all in liquid H-PU medium.

Determination that presence of the transforming plasmids conferred the selected phenotype

Two approaches were taken to show that the presence of the transforming plasmids was responsible for complementing the Δ*spe1* in each of the four transformed strains. First the plasmids were 'cured' from the strains. To accomplish this, each of the four transformant strains was grown in liquid non-selective medium, YEPD, for approximately eight generations. Each of these cultures was then streaked on YEPD agar. Single colonies were transferred to fresh YEPD agar and then tested for growth on YNBD-histidine agar and H-PU agar supplemented with uracil. All colonies lacking the ability to grow without uracil supplementation (indicating they lacked the vector-encoded *URA3* marker) also lacked the ability to grow without polyamine supplementation after depletion of internal polyamine stores. This indicates that complementation of the *spe1*Δ is plasmid-encoded and that loss of the transforming plasmid simultaneously resulted in loss of complementation.

The second approach taken was to re-transform Y359 with plasmid DNA isolated from the four transformants. Plasmid DNA isolated from Y359-2, -6, -9, and -13 was transformed into *E. coli*

© 1997 John Wiley & Sons, Ltd.

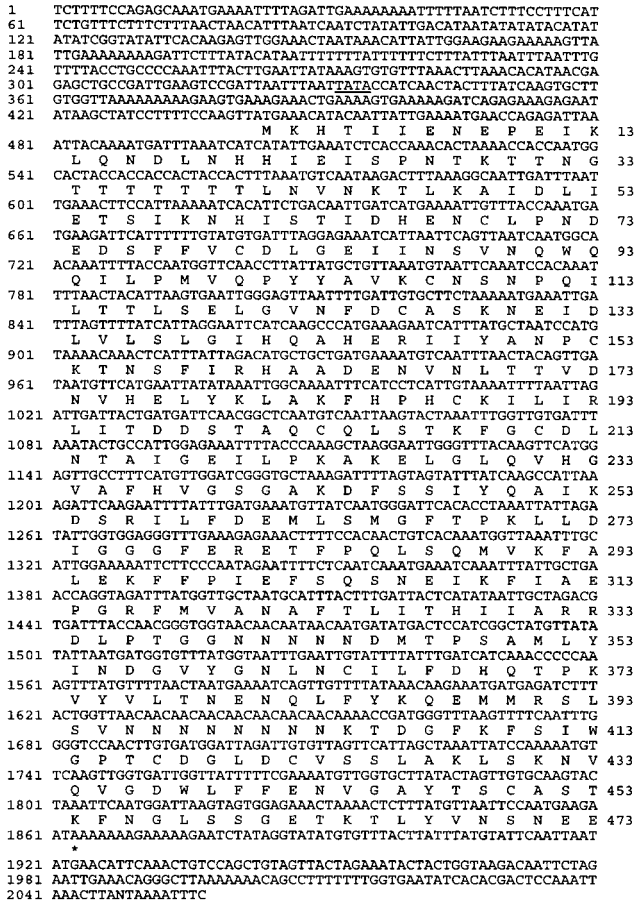

Figure 1. The nucleotide sequence and predicted amino acid sequence of the *SPE1* gene of *C. albicans*, encoding ornithine decarboxylase. A putative TATA box is underlined. The sequence contains two of the alternate CTG codons which normally code for leucine, but in *C. albicans* code for serine (amino acid residues 62 and 394). The *SPE1* sequence data are available from the GenBank library (accession number U85005).

DH5α to recover each plasmid. The plasmids, termed pY359-2, pY359-6, pY359-9, and pY359-13, were then transformed back into Y359. The selection of these transformants was identical to the selection used for the original transformant strains. Each of the plasmids was able to confer growth on H-PU medium to the secondary transformants. Additionally, the transformants receiving pY359-6 again showed slow growth on H-PU agar and no growth in liquid H-PU medium. The ability of the isolated plasmids to confer growth on H-PU medium to the Y359 strain indicates that the originally selected transformants did not bypass the *spe1* deletion, by virtue of a host mutation, but that this complementation is plasmid encoded.

Sequence analysis of the putative SPE1 *gene from* C. albicans

To determine whether the complementation technique successfully selected for an ODC-encoding gene, a plasmid from one of the wild-type-like transformants was selected for sequencing. An *Eco*RI fragment of approximately 3 kb from pY359-2 was subcloned into the low-copy number plasmid pACYC184 for initial sequencing. One end of this *Eco*RI fragment contained a

 © 1997 John Wiley & Sons, Ltd.

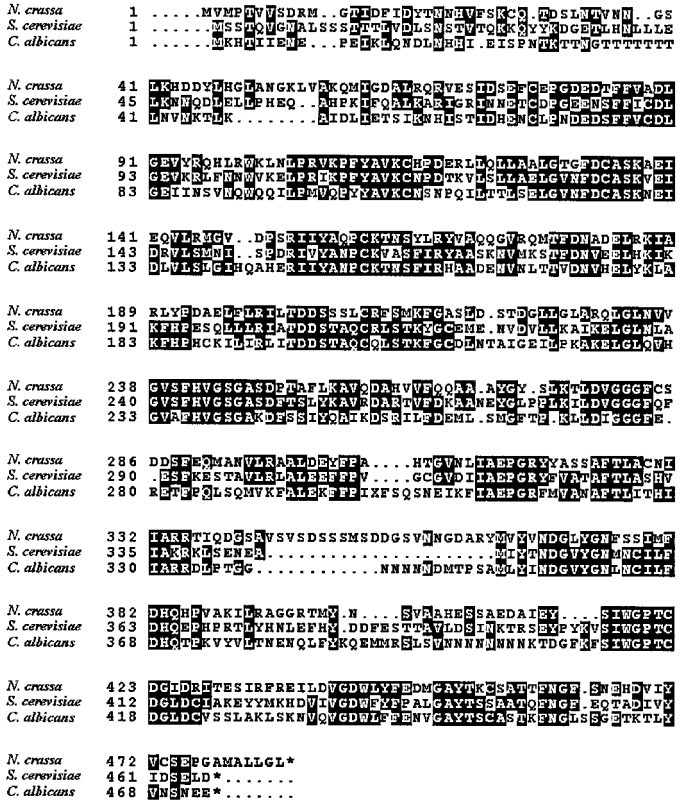

Figure 2. Amino acid sequence alignment of the ODC of *N. crassa*, *S. cerevisiae* and *C. albicans*. Alignment of the predicted amino acid sequences was performed using the GCG PILEUP program and drawn by the Box-Shade program. Residues conserved in at least two of the three proteins are shown as boxed positions. The *C. albicans* sequence is 47·3% identical (65·8% similarity) to the *S. cerevisiae* sequence and 39·9% identical (60·1% similarity) to the *N. crassa* sequence.

sequence with high homology to the *S. cerevisiae SPE1* gene (>65%). This initial sequence was used to develop sequencing primers for further sequencing and allowed us to determine that the pY359-2 insert contained an intron-free ORF of 1419 bases encoding a protein of 473 amino acids which shows extensive homology to the *SPE1* gene from both *S. cerevisiae* (Fonzi and Sypherd, 1987) and *N. crassa* (Williams *et al*., 1992).

The DNA sequence and predicted amino acid sequence are shown in Figure 1. An alignment and comparison of the predicted amino acid sequences of the ODC protein from these three fungi is shown in Figure 2. The alignment of the three fungal ODC proteins shows that the *C. albicans* predicted amino acid sequence contains the highly conserved sequences seen in most eukaryotic ODCs (Grishin *et al*., 1995). The *C. albicans* ODC protein also aligned well with the ODC proteins from a variety of organisms representing the other three eukaryotic kingdoms. Based on the extensive homology at both the nucleic and amino acid levels to other fungal ODC genes and proteins and the ability of the pY359-2 plasmid to restore growth to an ODC-negative strain of *S. cerevisiae*, we are confident that the gene encoding ODC from *C. albicans* has been isolated as reported here. In accordance with the rules of gene nomenclature for newly isolated *C. albicans* genes, we name the gene, *SPE1*.

Southern blot analysis of the four transforming plasmids

To test the nature of the inserts in the three other transforming plasmids capable of *spe1*

© 1997 John Wiley & Sons, Ltd.

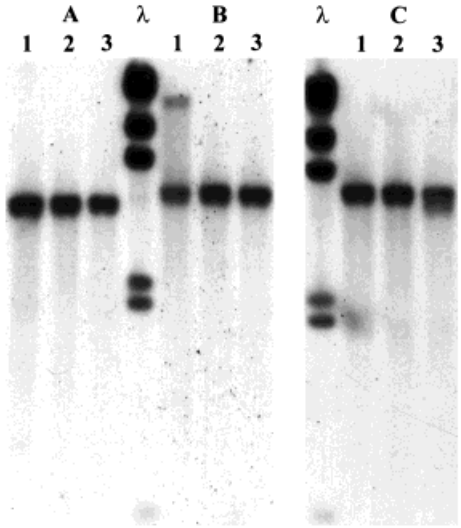

Figure 3. Southern hybridization of genomic DNA of three strains of *C. albicans* with a *SPE1* probe. Genomic DNA was isolated from B311, V6, or V6R (lanes 1, 2, 3, respectively), digested with restriction enzymes and run on an 0·8% agarose gel. The *Eco*RI-*Xba*I fragment of the *C. albicans SPE1* gene was used as a probe. (A) Lanes 1–3: *Eco*RV/*Eco*RI double digestion. (B) Lanes 1–3: *Eco*RV/*Pvu*II double digestion. (C) Lanes 1–3: *Hin*dIII/*Pvu*II double digestion. Lane λ: λ *Hin*dIII size markers.

complementation, Southern blot analysis of pY359-2, -6, -9, -13, and pSS1041 was performed by standard protocols using an *Eco*RI-*Xba*I fragment of the *SPE1* gene from pY359-2 as a hybridization probe. This probe hybridized to pY359-2, pY359-9, and pY359-13, but did not hybridize to pY359-6 or pSS1041 (data not shown). The lack of binding to pSS1041 indicates that the probe is specific for the *C. albicans* insert in pY359-2. These data indicate that pY359-9 and pY359-13 also contain the *SPE1* gene, but that pY359-6 contains a gene other than *SPE1*. The sequence contained in the pY359-6 insert will be the subject of a second report.

Southern blot analysis of C. albicans *genomic DNA*

After isolating the gene, we were interested in determining if *SPE1* exists as a single gene in *C. albicans*. The *SPE1* gene was used as a probe to look for the presence of homologous sequences in the *C. albicans* genome. Genomic DNA of the three strains of *C. albicans*, B311, V6 and V6R, was digested with restriction enzymes and probed with the *Eco*RI-*Xba*I *SPE1* fragment (Figure 3). In all cases except one, a single band was detected. In genomic DNA from strain B311 cut with *Eco*RV and *Pvu*II (Figure 3B), a second, larger band was also present. While this might be due to incomplete digestion of the DNA, it is more probable that this demonstrates the presence of a restriction enzyme polymorphism for one of the two enzymes used, located at a site outside of the *SPE1* gene. This would result in two bands of differing sizes in this diploid strain. In this regard, strain B311 has previously been shown to contain at least one chromosomal translocation (Thrash-Bingham and Gorman, 1992). In addition, restriction polymorphism for many genes has been reported for this diploid fungus and appears to be a common feature of *C. albicans*. The data suggest that only a single *SPE1* gene is present and that other genes with homology to *SPE1* are not present in the *C. albicans* genome.

Having isolated the gene encoding ODC, *SPE1*, from *C. albicans*, we can now use this sequence as a probe to examine expression of the gene during the morphological transition as well as to study the effects of its over- and under-expression. This should help elucidate the potential role of this enzyme in growth, differentiation and/or pathogenicity of this organism.

ACKNOWLEDGEMENTS

We thank Dr David Balasundaram for his gift of the Y359 strain and for early technical assistance. We also thank Dr Stuart Scherer for his gift of the *C. albicans* library. The editorial assistance of Gregory Harvey is greatly appreciated.

REFERENCES

Ausubel, F. M., Brent, R., Kingston, R. E., *et al.* (1993). *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

Balasundaram, D., Xie, Q.-W., Tabor, C. W. and Tabor, H. (1994). The presence of an active S-adenosylmethionine decarboxylase gene increases the growth defect observed in *Saccharomyces cerevisiae* mutants unable to synthesize putrescine, spermidine, and spermine. *J. Bacteriol.* **176,** 6407–6409.

Buckley, H. R., Daneo-Moore, L., Ahrens, J. C. and Sobel, J. D. (1986). Isolation of a germ-tube-forming revertant from *Candida albicans* B311V6. *Infect. Immun.* **53,** 13–15.

Buckley, H. R., Price, M. R. and Daneo-Moore, L. (1982). Isolation of a variant of *Candida albicans*. *Infect. Immun.* **37,** 1209–1217.

 © 1997 John Wiley & Sons, Ltd.

Cutler, J. (1991). Putative virulence factors of *Candida albicans*. *Annu. Rev. Microbiol.* **45,** 187–218.

Davis, R. H. (1990). Management of polyamine pools and the regulation of ornithine decarboxylase. *J. Cell. Biochem.* **44,** 199–205.

Fonzi, W. A. and Sypherd, P. (1987). The gene and primary structure of ornithine decarboxylase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **262,** 10127–10133.

Goshorn, A. K., Grindle, S. M. and Scherer, S. (1992). Gene isolation by complementation in *Candida albicans* and applications to physical and genetic mapping. *Infect. Immun.* **60,** 876–884.

Grishin, N. V., Phillips, M. A. and Goldsmith, E. J. (1995). Modeling of the spatial structure of eukaryotic ornithine decarboxylases. *Protein Science* **4,** 1291–1304.

Inderlied, C. B., Cihlar, R. L. and Sypherd, P. S. (1980). Regulation of ornithine decarboxylase during morphogenesis of *Mucor racemosus*. *J. Bacteriol.* **141,** 699–706.

Kaiser, C., Michaelis, S. and Mitchell, A. (1994). *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Martinez, J. P., Lopez-Ribot, J. L., Gil, M. L., Sentandreu, R. and Ruiz-Herrera, J. (1990). Inhibition of the dimorphic transition of *Candida albicans* by the ornithine decarboxylase inhibitor 1.4-diaminobutanone: alterations in the glycoprotein composition of the cell wall. *J. Gen. Microbiol.* **136,** 1937–1943.

Odds, F. (1988). *Candida and Candidosis: A Review and Bibliography*. Bailliere Tindall, London.

Odds, F. (1994). *Candida* species and virulence. *ASM News* **60,** 313–316.

Sambrook, J., Fritsch, E. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sherwood, J., Gow, N. A. R., Gooday, G. W., Gregory, D. W. and Marshall, D. (1992). Contact sensing in *Candida albicans*: a possible aid to epithelial penetration. *J. Med. Vet. Mycol.* **30,** 461–469.

Tabor, C. W., Tabor, H., Tyagi, A. K. and Cohn, M. S. (1982). The biochemistry, genetics, and regulation of polyamine biosynthesis in *Saccharomyces cerevisiae*. *Fed. Proc.* **41,** 3084–3088.

Thrash-Bingham, C. and Gorman, J. A. (1992). DNA translocations contribute to chromosome length polymorphism in *Candida albicans*. *Curr. Genet.* **22,** 93–100.

Williams, L. J., Barnett, G. R., Ristrow, J. L., Pitkin, J., Perriere, M. and Davis, R. H. (1992). Ornithine decarboxylase gene of *Neurospora crassa*: isolation, sequence, and polyamine mediated regulation of its mRNA. *Mol. Cell. Biol.* **12,** 347–359.

© 1997 John Wiley & Sons, Ltd.