Cloning, analysis and one-step disruption of the *ARG5,6* gene of *Candida albicans*

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The *ARG5,6* gene from the dimorphic fungus *Candida albicans* was cloned by functional complementation of the arginine auxotrophy present in strain EL2 (*Arg*[−]) using a gene library constructed in the double autonomously replicating sequence vector pRM1. Sequence analysis revealed a putative 857 amino acid polypeptide (95 kDa) which showed high homology (63% protein identity) to the *Saccharomyces cerevisiae* *ARG5,6* gene. Similarly to the *S. cerevisiae* gene, the *C. albicans* *ARG5,6* gene is responsible for both the acetylglutamate kinase and acetylglutamyl-phosphate reductase activities, the second and third steps of arginine biosynthesis at the mitochondria. The *C. albicans* *ARG5,6* gene complemented the *arg6* mutation present in *S. cerevisiae* (strain D160-4D) on a yeast episomal plasmid using its own regulatory signals. A set of non-integrative high-efficiency plasmid vectors based on this gene marker was constructed and a null *C. albicans* *arg5,6Δ* strain was obtained using the common *URA3*-blaster strategy. In addition, we generated an *arg5,6Δ* null mutant in a single transformation event, thus improving the basic strategy for generating gene deletions in *C. albicans*.

Keywords: *ARG5,6*, *Candida albicans*, molecular biology, gene disruption, arginine

INTRODUCTION

Despite the importance of *Candida albicans* as a model pathogenic yeast, obtaining information about its genetics has been difficult due to its diploidy and lack of sexual cycle (Kurtz *et al.*, 1990; Scherer & Magee, 1990). Although *C. albicans* genetics have largely relied on *Saccharomyces cerevisiae* as an intermediate genetic host, some of the most interesting and peculiar processes in *C. albicans*, such as its ability to switch between a yeast and a mycelial form of growth and its pathogenicity to humans (Odds, 1988), cannot readily be studied vicariously through a knowledge of *S. cerevisiae*. The development of an efficient transformation system, with the construction of both autoreplicative plasmids and suitable host strains, is then one of the major goals to facilitate genetic analysis of the biology and pathogenicity of *C. albicans*.

Although Kurtz and colleagues described both the integrative transformation (Kurtz *et al.*, 1986) and the

development of autoreplicative plasmids (Kurtz *et al.*, 1987) in *Candida*, plasmids often multimerize in *C. albicans* leading to difficulties in their isolation (Goshorn *et al.*, 1992). We recently developed plasmids carrying two independent autonomously replicating sequences (Cannon *et al.*, 1990; Herreros *et al.*, 1992) that allow the introduction and recovery of DNA in *C. albicans* (Pla *et al.*, 1995). A second important tool in *C. albicans* molecular genetics is the availability of isogenic auxotrophic host strains. Although both UV-enhanced mitotic recombination (Kelly *et al.*, 1987; Sadhu *et al.*, 1992) and toxic metabolites (Gorman *et al.*, 1991) have been used for gene disruption in *C. albicans*, the most commonly employed system now used makes use of the strategy adapted by Fonzi & Irwin (1993), which requires two sequential steps of gene disruption using the *C. albicans* *URA3* gene flanked by a heterologous *hisG* sequence from *Salmonella typhimurium*. This process could, however, be shortened if different genetic markers and appropriate strains were available for sequential disruptions. Although some nutritional genes have been isolated (Rosenbluh *et al.*, 1985; Goshorn *et al.*, 1992; Hoyer *et al.*, 1994; Pla *et al.*, 1995), there are no reports of strains with more than one auxotrophic marker obtained entirely by gene disruption.

The EMBL accession number for the nucleotide sequence reported in this paper is X98880.

In this work, we describe the isolation of the ARG5,6 gene from *C. albicans* by complementation of a mutagenized *C. albicans* arginine auxotrophic strain and its use in developing a genetic transformation system. In addition, we have constructed a triple *ura3Δ his1Δ arg5,6Δ* strain and demonstrate the generation of a homozygous *arg5,6Δ* null strain in a single transformation event.

METHODS

Strains and growth conditions. *C. albicans* and *Escherichia coli* strains are listed in Table 1. *C. albicans* strain 1001 (ATCC 64385), a wild-type strain from the Spanish Type Culture Collection, was used as the source of genomic DNA for the construction of a genomic library in plasmid pRM1 (Pla *et al.*, 1995). The following *S. cerevisiae* strains, obtained from the Yeast Genetic Stock Center (Berkeley, CA, USA), were used to check the function of the cloned *C. albicans* ARG5,6 gene by complementation: X3163-4C (MATa *arg1 met1 trp3 ade5 ura3 leu1 mal gal2*); STX63-8B (MATa *arg2 lys4 lys11 trp4 ade2 tyr1 rad2 gal2*); STX9-1A (MATa *arg3 ade2 gal2*); AB18-20A (MATa *arg4-8 ade2 ade5 CUP1⁺ leu2-3, 112 ura3-52 met13 cyh^r*); D160-4D (MATa *arg6 ura3 hom3 his1 trp2 ade1 met1 gal2*); X1049-9C (MATa *arg8 trp1 ura3 his3 asp5*); XJB17-2 (MATa *arg9 thr4 leu2 ade6 gal2*); and STX14-1C (MATa *arg10 ade2 gal2*). Yeast strains were grown in either YED medium (1%, w/v, yeast extract; 2%, w/v, glucose) or SD minimal medium (0.67% Yeast Nitrogen Base without amino acids; 2%, w/v, glucose), supplemented with uridine, histidine or arginine at 25 µg ml⁻¹, depending on the nutritional requirements, with continuous shaking at 30 °C. *C. albicans* Ura⁻ revertants were selected on 5-fluoroorotic acid plates as described previously by Navarro-García *et al.* (1995). *E. coli* strains were grown in Luria-Bertani (LB) or Terrific Broth (TB) at 37 °C supplemented with 100 µg ampicillin ml⁻¹ for plasmid selection.

DNA manipulations. All DNA manipulations were carried out following standard procedures (Sambrook *et al.*, 1989). Southern hybridization analysis was carried out using the Nonradioactive Labelling and Detection kit (Boehringer Mannheim) under high-stringency conditions on positively charged nylon membranes. For the determination of the ARG5,6 sequence, a 3.8 kb *Bam*HI–*Bam*HI insert from plasmid pAN1 was purified from agarose gels and sonicated to generate random fragments (300–600 bp in size) which were subcloned into the *Sma*I site of pUC19. Plasmid DNA was purified from *E. coli* transformants with Qiagen (Diagen) and both strands were sequenced with an Automated Sequencer (ALF; Pharmacia) according to Sanger *et al.* (1977) using the Universal and Reversal fluoresceinated primers. Sequence comparisons and homologies were carried out using the FASTA algorithm (Pearson & Lipman, 1988). DNA sequence analysis was performed with the PCGENE software.

Plasmid constructions and gene disruption. Plasmid pRM1, an efficient non-integrative plasmid, as well as its derived gene library have been described before (Pla *et al.*, 1995). The *E. coli*/yeast shuttle vector YEp352 has also been described previously by Hill *et al.* (1986). YEpARG was obtained by subcloning a 3.8 kb *Bam*HI–*Bam*HI fragment from pAN1 into the *Bam*HI site of YEp352. Plasmid pAN8, an autoreplicative plasmid which transforms *C. albicans* with high efficiency [$>5 \times 10^3$ transformants (µg DNA)⁻¹] and which is representative of a set of autoreplicative plasmids bearing this marker, was obtained by replacing the *Nae*I–*Sma*I fragment of pRM10 (Pla *et al.*, 1995) with a blunt-ended *Bam*HI–*Bam*HI fragment from pAN1. For the disruption of the *C. albicans* ARG5,6 gene, the same 4 kb *Bam*HI–*Bam*HI fragment was subcloned into pUC19 yielding pUC-ARG. An internal *Sna*BI–*Bgl*II fragment (which comprised most of the ARG6 domain) was then replaced with either the *Bam*HI–*Bgl*II fragment of pCUB-6 (Fonzi & Irwin, 1993) (carrying the *hisG*–*URA3*–*hisG* cassette) or the *Kpn*I–*Ssp*I fragment from YEp-HISX (Pla *et al.*, 1995) (carrying the *C. albicans* *HIS1*

Table 1. Strains used in this work

Organism	Strain	Genotype/phenotype	Origin
<i>C. albicans</i>	1001*	Wild-type	Gil <i>et al.</i> (1988)
<i>C. albicans</i>	SC5314	Wild-type	Gillum <i>et al.</i> (1984)
<i>C. albicans</i>	EL2†	Arg ⁻ Ade ⁻	Gil <i>et al.</i> (1988)
<i>C. albicans</i>	CAI4	<i>ura3Δ::imm434/ura3Δ::imm434</i>	Fonzi & Irwin (1993)
<i>C. albicans</i>	1006	<i>MPA1 ser57 ura3 lys1 arg4‡</i>	
<i>C. albicans</i>	RM1000	<i>ura3Δ::imm434/ura3Δ::imm434 his1Δ::hisG his1Δ::hisG</i>	Unpublished
<i>C. albicans</i>	CNC40	(RM1000)§ <i>arg5,6Δ::hisG-URA3-hisG/ARG5,6</i>	This work
<i>C. albicans</i>	CNC41	(RM1000)§ <i>arg5,6Δ::hisG/ARG5,6</i>	This work
<i>C. albicans</i>	CNC42	(RM1000)§ <i>arg5,6Δ::hisG/arg5,6Δ::hisG-URA3-hisG</i>	This work
<i>C. albicans</i>	CNC43	(RM1000)§ <i>arg5,6Δ::hisG/arg5,6Δ::hisG</i>	This work
<i>C. albicans</i>	CNC44	(RM1000)§ <i>arg5,6Δ::hisG-URA3-hisG/arg5,6Δ::HIS1</i>	This work
<i>E. coli</i>	MC1061	<i>araD139 Δ(ara-leu)7697Δ (lac)X74 galU galK traA</i>	Navarro-García <i>et al.</i> (1995)
<i>E. coli</i>	DH5αF'	<i>K12 Δ(lacZYA-argF)U169 supE44 thi1 recA1 endA1 hsdR17 gyrA relA1 (φ80lacZΔM15) F'</i>	Hanahan (1988)

* A wild-type strain from the Colección Española de Cultivos Tipo (Spanish Type Culture Collection), ATCC 64385.

† An Arg⁻ Ade⁻ auxotroph derived from strain 1001.

‡ Designated *arg57* (Goshorn & Scherer, 1989) and later shown to be *arg4* mutant (Hoyer *et al.*, 1994).

§ RM1000 background.

gene) yielding, respectively, pUC-ARG-U and pUC-ARG-H. Homologous integration of these constructs was achieved by transformation using DNA that had been linearized with *ScaI* and *BamHI*. The genotype of all strains was checked by Southern analysis using a *Clal*-*ScaI* fragment of *C. albicans* ARG5,6 as a probe and digesting the genomic DNA of the transformants with *HpaI*-*Clal* (Fig. 1).

Genetic transformation procedures. *C. albicans* was transformed using the standard protoplast method with minor modifications (Herreros *et al.*, 1992; Pla *et al.*, 1995). Gene disruption of *C. albicans* ARG5,6 by cotransformation of pUC-ARG-U and pUC-ARG-H was optimized with respect to the concentration of both DNAs used in the assay. Protoplast fusions were performed as described previously (Gil *et al.*, 1988). Electroporation was used for the recovery of plasmid DNA from *C. albicans* transformants (Pla *et al.*, 1995).

RESULTS AND DISCUSSION

Isolation of the *C. albicans* ARG5,6 gene

Strain EL2 is an arginine and adenine auxotroph obtained through ethylmethane sulfonate mutagenesis of strain 1001 as described previously (Gil *et al.*, 1990). This auxotrophic mutant was used for the generation of a set of *C. albicans* mutants altered in their ability to undergo the dimorphic transition (Gil *et al.*, 1988, 1990). We first verified that the arginine biosynthetic defect present in strains EL2 and 1006 (*arg4*; Hoyer *et al.*, 1994) was different since it was possible to isolate prototrophic

hybrids when parasexual crosses between both strains were carried out. A gene library constructed in the double autonomously replicating sequence vector pRM1 (Pla *et al.*, 1995) was used to isolate the complementing gene. Screening of approximately 50000 transformants yielded 14 prototrophs after 7 d growth in selective medium. Only one of them was characterized further. Plasmid DNA, designated pAN1, was extracted from this transformant and was shown to be capable of complementing the auxotrophic phenotype upon retransformation. Standard restriction analysis revealed an insert of approximately 7 kb, with a minimal 3.8 kb *BamHI*-*BamHI* complementing region (Fig. 1). When present in the episomal plasmid YEp352 (plasmid YEp-ARG), this DNA was shown to complement the *arg6* defect present in strain D160-4D (an *arg6* mutant). These results demonstrate the usefulness of these replicative plasmids (Cannon *et al.*, 1992; Pla *et al.*, 1995) in *C. albicans* genetic manipulation.

Sequence analysis of the *C. albicans* ARG5,6 gene

Sequence analysis of the insert revealed a putative ORF of 857 amino acids (95 kDa). This protein was 63% identical to the predicted translation product of the ARG5,6 gene of *S. cerevisiae* (54% identical to *Schizosaccharomyces pombe* Arg5,6p), thus indicating that we had cloned the *C. albicans* ARG5,6 gene. In *S. cerevisiae*, this gene is responsible for the generation of a polypeptide which is post-translationally processed yielding two mature polypeptides that encode the acetylglutamate kinase (ARG6) and acetylglutamate-phosphate reductase (ARG5) activities in the arginine biosynthetic pathway (Boonchird *et al.*, 1991). Thus, in *C. albicans*, the genetic organization of the ARG5,6 locus is apparently similar to that in *S. cerevisiae* (Boonchird *et al.*, 1991) and *Schiz. pombe* (van Huffel *et al.*, 1992) and contrasts with the situation in prokaryotes where each activity is encoded by a separate gene. A putative TATA box was found around position 253 (Fig. 2), while a putative transcription termination signal was at 2943–2967 (based on their homology to *S. cerevisiae* consensus signals). No obvious *S. cerevisiae* GCN boxes (consensus sequence TGACTC) could be found in the 5'-upstream region, as has been observed for the *C. albicans* ARO3 (Pereira & Livi, 1995) and ARG4 (Hoyer *et al.*, 1994) genes. In addition to the *arg4* mutant strains 1006 and TMSU221, another complementation group designated *arg100* has been described for strains A642, hOG318, hOG357, FC18-6 and WC-5-4 (Hoyer *et al.*, 1994) located on the R chromosome. pAN8, an auto-replicative plasmid bearing the ARG5,6 gene, did not complement hOG318 arginine auxotrophy.

Construction of an *arg5,6Δ* null mutant

We used two different strategies to obtain an *arg5,6Δ* mutant in the *ura3Δ his1Δ* strain RM1000 background. We first used the *URA3*-blaster protocol to generate an *arg5,6Δ* mutant. Using the *URA3* marker (construction

Fig. 1. Physical map of the ARG5,6 locus and its disruption strategy. The *hisG*-*URA3*-*hisG* cassette and the *HIS1* gene were used to replace an internal fragment of the *C. albicans* ARG5,6 gene. Only the relevant restriction sites are shown, with numbers indicating the position in kb. The asterisks indicate restriction sites that are derived from the multiple cloning site of the pUC polylinker and are not found in the genomic DNA. Arrows represent the structural genes. The probe used in Southern blot analysis and the recombination region are indicated (see Methods).

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ACTTTGAATATTTTAAACCATTAGTACTAATTCATATTGCTTTTGCTTTGATAAAAA 60
AAAAAAGAGGACGCAAAATTTGGTGAGGTGACGTGCACACTGATCTTACATCGCGGTAAAG 120
AAAAATTTTCCAAAAATAATTACTCTTTTGTGATCAAAATTTATATTCAATTTAAATTTTG 180
CGATAATAATGAAATTTTAAAGGAGGACAAATTTAAATTTGTAAGAGAAA 240
ATTTTTCATATATATACCAAGTTTCTTTCTTTCTGCTGTTTGAACCTTTTCTTT 300
ATATATTTGATTGAGTCCCTTATCTGCTTATAATGATCAGACAAGTGAATAAAAAGG 360
M I R Q V N K K

CTATTTCAACAGCTCTTTTCAAAAGATTATCGCTCAGCGGTAGTGTCTTTGCTAATATTA 420
A I S N S L F K R L S L S G S A F A N I

CAGCCAATAAAAAATCGTCCACTCATCACTCAACCAAGACTCAATTAGCAAAATGTTTC 480
T A N K K S S T H Q L A N V

GTTTCTATTCAACAAATCCACTGTTATTCAGTTTATGAACAACATTTGCTTCCAAACGTG 540
R F Y S T K S T V I Q L L N N I G S K R

AAGTTGAACAATACTTGAATATTTCACTTCGGTATCTCAACAACAATTTGCTGTGATCA 600
E V E Q Y L K Y F T S V S Q Q Q F A V I

AAGTTGGTGTGCAATATCACCAACAATTTGAACGAATTTAGCATCATGCTTGGCATTTT 660
K V G G A I I T Q Q L N E L A S C L A F

TGTATCATGTTGGTTTGTACCTTATGTTTACATGGTACTGGTCCCAAAATCAATGAAT 720
L Y H V G L Y P I V L H G T G T P Q I N E

TGTGTGAAAACGAAGGTGTTGAACGAATATATGATGTTATGATGATCAACCAACCCCA 780
L L E N E G V E P E Y I D G I R I T N P

AAACAATGGAAGTAGTACGTAAGTGTCTTCTAGAGCAAACTTGGTGTGACAGCTT 840
K T M E V V R K C F L E Q N L R L V T A

TGGAAAAATTTGGTGTTCACGCTCGTCCAATTAACGCTGGTGTATTTGAAGCTGAATATT 900
L E K I G V H A R P I T A G V F E A E Y

TGGATAAGGACAAATATCAATAGTTGGTAAAACTACTAGTGTCAACAAATCCCAAGTTG 960
L D K D K Y Q L V G K I T S V N K S P V

AAGCTGCCATCAATAGTGGATCTTGCCTAATTTTGACATCTTTGGCCGAAACCTCTCTGG 1020
E A A I N S G Y L P I L T S L A E T S S

GTCAATATTAATATCAATGCGGACGTTGCTGCGGAGAAATTAGCTCGTGAATTTGAAC 1080
G Q L L N V N A D V A A G E L A R E F E

CTTTGAAAATTTGTTTACTTGAATGAAAAGGTGGTATTATTAACGGAACACTGGTGAGA 1140
P L K I V Y L N E K G G I I N G N T G E

AAGTTTCTGCTATCAATTTGGATGAAGAATATGAAGATTGTTTAAAGGAAAGTTGGGTTA 1200
K V S A I N L D E E Y E D L L K E S W V

AATATGGTACTAAATGAAGATCAAGGAAATTCACGATTTATTACAACATTTGCCAAGAT 1260
K Y G T K L K I K E I H D L L Q H L P R

CTTCTTCAGTTGCCATTTATGATGTCAACGATTTTCAAAAGGAATTTGTTCACTGATTCTG 1320
S S S V A I I D V N D L Q K E L F T D S

GTGCTGGTACTTTAATCAGAAGAGGTACAGATTAATTAACAGAACTCTTTCGGTGATT 1380
G A G T L I R R G Y R L I N R N S L R D

TTGGTAACCCAGACTTGTAAAGAACGCATTTTAAAGAGCCAGAAATCAAAATCGGTA 1440
F G N P D L L R N A L L R D P E I K T G

AAGTGTCTGTTGCTTCACTTGAAGTTTGGATCTGTTCAATTCAGAGTTATGGAG 1500
K V S A Y L K F L D S V Q F K S Y G

ACGAACCTTTGGAAGTATTTGGCTATTTGGTGTAGAACAAATGACAAATTCCTAAATAG 1560
D E P L E V L A I V V E Q N D K I P K L

ACGAGTTTGTCTCAAGACAGGTGGTTAAACAAATGTTACCGATAATATTTTCAATG 1620
D E F L S S K T G W L N N V T D N I F N

CTATCAAGAAAGATTACAGTCAATATTGTTGGGTGTTAATGAAAACGATGCCAACTTAC 1680
A I K K D Y S Q L C W V V N E N D A N L

CTTGGTATTCTCCAAATCAGATGGTTTCATTGGCCAAGATGGCCAAATCTTGTGTTGGT 1740
F W Y F S K S D G S F A K N G Q I L F W

ACGGTTTAAACATTGACGAAGCTAGTAAATTGATTAAAGAAATTTGATTCTTCTATCTATTG 1800
Y G L N I D E A S K L I K E F D S S S I

GATCATCGTTGTCTCTTCTAAGAAATCTGGCGTATTACATCTGCTCAACAAAGCGTG 1860
G S S L S S S K E S G V F T S A Q Q K R

GTTTCCCACTCTACAGTCCGTAGAACACCAATCTTATCTTCCATTATCTGAAGGTA 1920
G F H H S T V R R N T N P N P P L S E G

AGCAAAACGAGAGAAAAAAGTTGCTTTGATTGGTGTGATAGGTTTACACTGACAAAAC 1980
K Q T E R K K V A L I G A R G Y T G Q N

TGATTAATTTGATGACAACTCATCACTTTGGATATTTATATGATGCTGCTCAGTGAAT 2040
L I K L I D N H P Y L D I S Y V S S R E

TGGAAAGTCAAAAATTTACAAGGTTTACAACAAAGATAACATTTGTTTACTTCAACTTGCAA 2100
L E G Q K L Q G Y N K D N I V Y S N L Q

TTGAAGACATCAAGAGATTAGAAGAAAAAATGAGGTTGATGTTTGGGTTATGGCTTTAC 2160
I E D I K R L E E N N E V D V W V M A L

CTAATGGTGTGTTTGAACCAATTTGTTGACACTATTGATTGTTTGTGATCAGAACCAATCT 2220
P N G V C K P F V D T I D L V Q N P N S

AAATTTGTCGATTTATCTGCTGATTTATGTTTGAACAACTGTTGAATGGACTTTATGGAT 2280
K I V D L S A D Y R F D T T G E W L Y G

TACCAGAACTAAATGATCGTAAGACCATAGCTCAAGCTAAGAAAATTTTCAACCCAGGTT 2340
L P E L N D R K T I A Q A K K I S N P G

GCTATGCCACTGCTGCTCAAGTCCCATTTGCCCGTTTAAAGAAATATATTTCTGGAAC 2400
C Y A T A A Q V A I A P L K E Y I S G T

CAAGCATTTTGGTGTGTTCTGGCTATTTCAGGTGCTGGAACCAACCATCTCCGAAGAACG 2460
P S I F G V S G Y S G A G T K P S P K N

ACGTCAATTTATTTGCAAAATTTAATTCCTTACTGTTTAACTGATGATGATGACAGAAA 2520
D V N L L S N N L I P Y S L T D H V H E

AAGAAATAGTAGTCAATTAGGTTTACAAGTTGACATTTACCCACAGTGTGCTCAATGGT 2580
K E I S S Q L G L Q V A F T P H V A Q W

TTCAAGGTTATTTACTACTATCAACATTTCCAAATTAAGAAAGGATCTTTGACTTCTCGTG 2640
F Q G I T H T I N I P I K K G S L T S R

AAATTAGAAACATTTACCAAGATAGATATCAAGGTGAAAAGCTAATCACCATTAGTGAG 2700
E I R N I Y Q D R Y Q G E K L I T I S G

AAGCACCCTTGTGTTAAGACATTTAGTGGCAACACGGTGTGCTGTTGTTGGTGGCTTTG 2760
E A P L V K D I S G K H G V V V G G F A

TCAACTCTAATGAAGACCGTGTGTTATTGTTGTCCTATTGACAAATTTGTTGAAGAGTG 2820
V N S N E D R V V I V A T I D N L L K G

CAGCTACCAATGTCTACAAAATATAAATTTGAGTCAAGAAATTTGGTGAATACGATGGTA 2880
A A T Q C L Q N I N L S Q E F G E Y D G

TTCCAACCGAATCTTGTATCAGAGGCTAAACTTGCACAAAAGAGTATGAAATTTTATA 2940
I P T E S L I R G -

GATAGTTATTTATGTTAGTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT 3000
TCATATACACTGTTGTTGATTAGATTAAAGTTTGTATATTTCTTCTTCTTCTTCTTCTTCT 3060
AATATTTTATAGCAATTAAGATCC

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Fig. 2. Nucleotide sequence of a *Scal*-*Bam*HI fragment encoding *C. albicans* ARG5,6 with its deduced amino acid translation. The presumed TATA box region (underlined) and the putative transcription termination signal (double underlined) are shown. *Scal* and *Bam*HI sites are underlined in bold.

pUC-ARG-U), a clone was selected which was shown by Southern blotting to integrate the *URA3*-blaster construction at the ARG5,6 locus, yielding the heterozygous strain CNC40. Excision of *URA3* was achieved on 5-fluoroorotic acid plates, yielding strain CNC41. Strain CNC42 (homozygous *arg5,6Δ* strain) was obtained through a second round of transformation which allowed us to obtain arginine auxotrophs at a frequency of 15%. Finally, excision of *URA3* on 5-fluoroorotic

acid plates yielded strain CNC43 (*ura3Δ his1Δ arg5,6Δ*). In an alternative approach, the *C. albicans* *HIS1* gene (Pla *et al.*, 1995) was used to replace an internal region of *C. albicans* ARG5,6. Strain CNC40 was transformed with pUC-ARG-H making use of *HIS1* (see Methods) as a selectable marker (Fig. 1). Fifteen of 21 Ura⁺ His⁺ transformants (70%) were shown to be arginine auxotrophs. This strain was efficiently transformed to arginine prototrophy with plasmid pAN8 (see Methods).

The availability of an appropriate genetic marker, the *C. albicans* ARG5,6 gene, allowed us to check the feasibility of a single-step gene disruption in *C. albicans*. We cotransformed strain RM1000 (*his1Δ ura3Δ*) with both URA- and HIS-blaster constructions. A cotransformation frequency of approximately 10% was found, similar to that quoted by other authors (Kurtz *et al.*, 1986, 1990). Ten per cent of the prototrophic clones were shown to be arginine auxotrophs (strain CNC44) carrying the correct (i.e. homologous) recombination event at the ARG5,6 locus. This result is in general agreement with estimated frequencies for single recombination events (70% and 15%).

We conclude that it is possible to generate a deletion in a specific *C. albicans* gene in a single transformation step. This should expedite the generation of homozygous null mutants by integrative transformation by reducing substantially the number of steps required to achieve the disruption of the two alleles at each genetic locus. Strains with different gene markers (like the *ura3Δ his1Δ arg5,6Δ* strain described in this work) and suitable for genetic transformation should allow the deletion of both alleles of a specific gene while maintaining the wild-type gene on an episomal plasmid.

ACKNOWLEDGEMENTS

We thank R. M. Pérez Díaz for her excellent technical assistance, F. Navarro-García for critical reading of the manuscript, M. L. Hernáez for helpful scientific comments and M. García Sáez for the expert sequencing technical work (Servicio de Secuenciación Automatizada de DNA de la Universidad Complutense). This investigation was supported by the grant FIS93/0183 from Fondo de Investigaciones Sanitarias. L. Monteoliva is the recipient of a fellowship from the Universidad Complutense de Madrid.

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Received 1 July 1996; revised 27 September 1996; accepted 8 October 1996.