Two transcripts, differing at their 3′ ends, are produced from the Candida albicans SEC14 gene

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A search for Candida albicans mutants defective in filamentous growth led to the isolation of a mutant strain with an insertion mutation in the SEC14 gene. SEC14 encodes the phosphatidylinositol/phosphatidylcholine transfer protein, an essential protein in the yeast Saccharomyces cerevisiae. In the dimorphic yeast Yarrowia lipolytica, SEC14 is needed for growth only in the hyphal form and is not required for growth in the yeast form. However, unlike Y. lipolytica SEC14, C. albicans SEC14 is probably essential for growth. Northern blot analysis and PCR amplification of transcripts produced from the SEC14 gene demonstrated that two transcripts differing at their 3′ ends were produced. The two transcripts may regulate the activity of SEC14 so that Sec14p can perform two functions in C. albicans. One function may be an essential function analogous to the function of Sec14p in S. cerevisiae and the second function may be important during filamentous growth, analogous to the function of Sec14p in Y. lipolytica.

Keywords: Candida albicans, SEC14, transcripts, restriction enzyme-mediated integration

INTRODUCTION

The opportunistic pathogen Candida albicans has become an increasingly important pathogen in recent years, particularly in hospital settings (Pfaller, 1994). Immune-compromised patients such as oncology patients and transplant recipients are susceptible to life-threatening infections by C. albicans. As a means of improving methods for treatment of such patients, increased understanding of the biology of C. albicans is important.

In this paper, an analysis of the C. albicans SEC14 gene is described. SEC14, encoding the phosphatidylinositol/ phosphatidylcholine transfer protein, has been extensively studied in Saccharomyces cerevisiae (Bankaitis et al., 1990). SEC14 is an essential gene in S. cerevisiae and its product is required for normal function of the protein secretory pathway. Bankaitis and coworkers have proposed that SEC14 acts as a sensor of lipid composition in the Golgi (McGee et al., 1994). Sec14p has been shown to regulate the activity of the enzyme choline-phosphate cytidylyltransferase and, thereby, to modulate the activity of the CDP-choline pathway for phosphatidylcholine biosynthesis in S. cerevisiae (Skinner et al., 1995). Recently, the C. albicans SEC14 gene was characterized (Monteoliva et al., 1996). C. albicans SEC14 was shown to be highly homologous to the S. cerevisiae SEC14 gene. In addition, it was suggested that C. albicans SEC14 may be an essential gene (Monteoliva et al., 1996).

In the dimorphic yeast Yarrowia lipolytica, SEC14 is not essential for growth when the cells are in the yeast form and it is not required for function of the protein secretory pathway (Lopez et al., 1994). Y. lipolytica sec14 mutants are viable but exhibit defects in filamentous growth. Thus, in this organism, SEC14 has a function that appears to be unrelated to the protein secretory pathway.

C. albicans is also a dimorphic yeast, capable of growth by budding as a yeast cell or of growth in the filamentous

Abbreviations: REMI, restriction enzyme-mediated integration; UTR, untranslated region.
† Deceased.
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forms, hyphae and pseudohyphae. These filamentous forms are preferentially observed in specimens taken from infected sites and it is generally believed that filamentous growth contributes to the virulence of C. albicans (Odds, 1994). In this study, a search for genes that were required for filamentous growth led to the isolation and study of the SEC14 gene. An analysis of SEC14 transcription revealed that two transcripts differing at their 3’ ends are produced from the SEC14 gene.

To isolate mutant strains defective in filamentous growth, restriction enzyme-mediated integration (REMI) was used as a means of mutagenizing the chromosome of C. albicans. REMI involves transformation of an organism with a linear fragment of DNA that is not homologous to its chromosome, in the presence of the enzyme used to produce the DNA fragment. In the presence of the enzyme, the transforming DNA becomes integrated into the chromosome in an appropriate restriction site. This procedure, first used in S. cerevisiae (Schiestl & Petes, 1991), has been used as a method of insertion mutagenesis in Dictyostelium discoideum (Kuspa & Loomis, 1992) and Ustilago maydis (Bolker et al., 1993). We have previously described stable integration of transforming DNA onto the chromosome of C. albicans cells using REMI (Brown et al., 1996). In this study, REMI was used as a method for producing insertional mutants and a strain containing an insertion in the SEC14 gene was isolated. However, further analysis showed that the insertion mutation is not responsible for the defect in filamentous growth exhibited by the mutant strain.

**METHODS**

**Strains and plasmids.** C. albicans strains SGY-243 (Aura3/Aura3) (Kelly et al., 1987) and CAI-4 (Aura3/Aura3) (Fonzi & Irwin, 1993) were used as the parents of all strains in this study. Strain IVS9 was isolated as a mutant defective in filamentous growth after mutagenesis of SGY-243 by REMI and UV treatment. IVS9 carries an insertion of pCK61 DNA in one copy of SEC14. Strains IVS10 and IVS11 were constructed from CAI-4 using the UrA-blastere method (Alani et al., 1987). CAI-4 was transformed by electroporation with pI57 after digestion of the plasmid with SpeI and HpaI. pI57 contains a deletion in the SEC14 locus extending from a BglII site upstream of SEC14 to the HindIII site within the SEC24 structural gene (Fig. 1). A BglII–HindIII fragment from pMB7 (Fonzi & Irwin, 1993), encoding C. albicans URA3 flanked by direct repeats of DNA encoding hisG from Salmonella typhimurium (the hisG-URA3-hisG Ura-blastere cassette), was cloned into this deletion. After transformation, Ura* colonies were selected. Transformants were screened by Southern blot analysis and a strain designated IVS10 (SEC14'/Asec14::hisG-URA3-hisG) was isolated. Ura* derivatives of strain IVS10 were selected by plating cells on medium containing 5-fluoroorotic acid (1 mg ml⁻¹) (Ausubel et al., 1989). The resulting Ura*, heterozygous SEC14 deletion strain was designated IVS11 and verified by Southern blot analysis.

Plasmid pCK61 (pBR322 carrying C. albicans URA3) has been described previously (Brown et al., 1996). pI52, isolated from the chromosome of strain IVS9, is pCK61 containing additional DNA derived from the SEC14 locus (Fig. 1). To create strain IVS9, pCK61 was digested with BamHI and integrated into the BamHI site of SEC14 by REMI. Digestion of IVS9 chromosomal DNA with the enzyme HindIII, which does not cleave within pCK61, followed by ligation and transformation of Escherichia coli, allowed the isolation of a plasmid (pI52) containing sequences derived from the chromosomal DNA flanking the site of insertion.

Clones encoding an intact copy of SEC14 were obtained by hybridization of a probe derived from pI52 to a plasmid library of SGY-243 genomic DNA fragments (D. Brown & C. Kumamoto, unpublished). Two of these clones, designated pI54 and pI56, contained inserts of approximately 3 and 5-5 kb, respectively, including the SEC14 gene (Fig. 1).

**E. coli** strain XL1-Blue (Stratagene) was used for plasmid production.

**Media.** Media for growth of C. albicans strains were YPD rich medium and CM-ura minimal medium, as described by Ausubel et al. (1989). Liquid minimal medium was NC glucose [Yeast Nitrogen Base without amino acids (Difco), 0.1% Casamino acids (Difco), 2% glucose], as described previously (Brown et al., 1996). To stimulate filamentous growth of strain SGY-243, cells were grown at 30 °C in YPD medium for 1–2 d until the cultures reached high culture density. The cells were washed and starved in water overnight. The cells were diluted 100-fold into GT1 medium (2% yeast extract, 2% glucose, 20 μg uridine ml⁻¹) or GT2 medium (0.4% yeast extract, 0.4% glucose, 4 μg uridine ml⁻¹) and incubated at 37 or 40 °C for 4 h. Both SGY-243 and CAI-4 produced germ tubes upon culturing in GT1 or GT2 medium at 37 or 40 °C.

For growth of E. coli, L broth (Miller, 1972) with or without 100 μg ampicillin ml⁻¹ was used.

**Transformation by electroporation.** Electroporation of C. albicans was performed essentially as described previously (Brown et al., 1996), except that the first two washes of cells were performed using water rather than 10% (v/v) glycerol. The last two washes of cells were performed using 10% glycerol. Cells were frozen in 50 μl aliquots at −80 °C. For transformation by REMI, an aliquot of cells was mixed with 18 μg BamHI-digested pCK61 DNA and 100 units BamHI (New England Biolabs). The sample was electroporated at 20 kV, 25 μF, 200 Ω in a 0.2 cm gap cuvette (Bio-Rad). The electroporated cells were diluted with chilled YPD and plated on CM-ura plates. Integrative transformation by electro-
poration was performed similarly except that BamHI was omitted and lower amounts of DNA were used.

Electroporation of E. coli was performed using a Bio-Rad Gene Pulser electroporator according to the manufacturer’s recommendations.

**Isolation of mutant IV89.** Strain SGY-243 was subjected to REMI mutagenesis with BamHI-digested pCK61. Approximately 8000 transformants were obtained in 10 independent pools. Cells from each pool were grown in NC glucose liquid medium, cells were spread on YPD plates and the plates were irradiated with UV light for 40 s. After incubation, cells were scraped off the plates and grown in liquid culture in YPD at 30 °C. After 1–2 d of growth, when the cultures had reached high density, the cells were washed with water, resuspended in water and starved overnight. Cells were diluted 100 fold into GT1 medium and incubated at 37 °C for approximately 4 h. Following this treatment, the cultures were filtered through Whatman No. 1 filter paper. YPD was added to the filtrate and the cells were grown in YPD at 30 °C to high cell density. The mutagenized pools of cells were cycled through this enrichment procedure 4–6 times. After the last round of enrichment, cells were plated and allowed to form colonies on YPD plates. Individual colonies were tested for their ability to initiate filamentous growth. One colony from each independent pool was retained for further study.

**DNA analysis.** *C. albicans* chromosomal DNA extraction was performed either as described previously (Brown et al., 1996) or using Qiagen columns, according to manufacturer’s instructions. Plasmid DNA extractions, restriction digestion, agarose gel electrophoresis and Southern blotting were performed by standard methods (Sambrook et al., 1989). DNA sequencing was performed using the Circumvent cycle sequencing kit (New England Biolabs) according to manufacturer’s instructions or by the DNA Sequencing Core Facility at the Tufts University Protein Analysis Facility. Oligonucleotides were synthesized by Mike Berne of the Tufts University Protein Analysis Facility. DNA fragments were amplified by PCR (Mullis et al., 1986) and PCR products were cloned using the Invitrogen TA cloning kit according to manufacturer’s instructions.

**RNA analysis.** For RNA extraction, cells were lysed using glass beads (Ausubel et al., 1989) and the lysate was extracted with phenol (Ausubel et al., 1989) or Trizol (Gibco BRL). Ten or twenty micrograms of total RNA per sample was separated on a formaldehyde/agarose gel, transferred to a Nyttran-plus membrane and probed by standard Northern blotting methods (Sambrook et al., 1989). To prepare probe B (Fig. 1), plS4 plasmid DNA encoding SEC14 was amplified with primer MTTM (5’–ATGACTGATGACTACTGAAGA), corresponding to the first 8 codons of SEC14, and primer 1 (5’–TGTTGTGTTGTGGTATGTATGTGGT; see Fig. 5), complementary to sequences contained in the SEC14 untranslated region (UTR). A single PCR product was obtained. Inverse PCR was used to prepare probe C (Fig. 1) as follows. Plasmid plS4 was digested with Sau3A and the fragments were ligated under dilute conditions. The ligated DNA was amplified with primers 5’–CTTATTAACATTTGC and 5’–CTAGGACATCC, corresponding to nt 127–143 (see Fig. 5) and the complement of 1222–1235 (Monteoliva et al., 1996), respectively, to amplify DNA downstream of the known SEC14 5’ end determined from cDNA analysis. The amplified DNA was cleaved with Sau3A to release sequences derived from the known 3’ UTR that were joined to the downstream sequence by ligation. Probes A and D (Fig. 1) were generated by digestion of plasmids plS4 and plS2, respectively. PCR products were purified using Qiagen spin columns. Fragments were labelled by random priming with [α-32P]dATP. Size standards were visualized by staining with methylene blue (Wilkinson et al., 1990). Blots were quantified using ImageQuant software (Molecular Dynamics) after either densitometric scanning of films or scanning of the blot using a Molecular Dynamics PhosphorImager. To calculate the ratio of the transcripts, the amount of the band representing the larger transcript was divided by the amount of the band representing the shorter transcript.

**Analysis of cDNA.** Total RNA was extracted from SGY-243 cells grown at 30 °C in YPD. PolyA+ RNA was purified from this RNA using the Invitrogen FastTrack Kit 2.0, according to manufacturer’s instructions. The RNA was reverse transcribed using primer oligo-dT (NXT4, where N is any of the four bases and X is A, C or G) as the primer (Ausubel et al., 1989). To amplify the coding sequence and 3’ UTR, the cDNA was amplified with primers MTTM and oligo-dT. Products of approximately the expected size, based on the amino acid sequence of Sec14p and expected sizes of the UTR, were cloned using the Invitrogen TA cloning kit. Clones containing SEC14 cDNAs were identified by colony hybridization (Sambrook et al., 1989). To amplify the 5’ UTR, the cDNA was incubated with dCTP and telomerase DNA polymerase and the products were amplified with the primers 5’–AGGAATTCCG, and either 5’–TTTGATCCTGGTGGAGC–GGTGTG or 5’–AGGTGTATATATGTGTAACC, corresponding to the complements of Sec14p residues 16–22 and 24–31, respectively. Amplified products were cloned as above.

To detect the second, longer SEC14 transcript, cDNA was amplified using primer MTTM and either primer 1, primer 2 (5’–ATTAAGGAAATTTCACG) or primer 3 (5’–GTATTTGTATGTGTCGTC), corresponding to the complement of nucleotides 24–47, 287–303 or 427–444 (see Fig. 5), respectively.

**RESULTS**

Restriction enzyme-mediated integration produces chromosomal insertions at a large number of sites

Previously, the ability of a restriction enzyme, BamHI, to stimulate non-homologous transformation of DNA into *C. albicans* was reported (Brown et al., 1996). Transformants were found to contain insertions of the transforming DNA at chromosomal BamHI sites. Since such transformation events could disrupt structural genes, it was possible that REMI could be used to mutate the *C. albicans* genome, if the insertions occurred without preference for BamHI sites. In a previous study, a small number of transformants were analysed and found to contain insertions of the transforming DNA at different BamHI sites (Brown et al., 1996).

To demonstrate that large numbers of sites could be used and that there were no preferred sites for integration, larger numbers of transformants were analysed. In this analysis, strain SGY-243 was transformed with BamHI-digested pCK61 in the presence of BamHI enzyme. Chromosomal DNA was prepared from two pools containing 50 Ura+ transformants and one pool containing 1000 Ura+ transformants. The DNA
Structure of the \textit{URA3} locus in SGY-243

Structure of a REMI insertion

Fig. 2. Analysis of the sites of plasmid integration in strains subjected to REMI. (a) A restriction map of the \textit{URA3} locus of strain SGY-243 is shown, with the portion that hybridizes to the probe indicated by the black box. A diagram of a hypothetical insertion mutation is shown below. The open box indicates pCK61 sequences and the black portion indicates the fragment that hybridizes to the probe. B, BamHI; C, Clal. (b) Southern blot of chromosomal DNA isolated from transformants constructed by transforming SGY-243 cells with BamHI-digested pCK61 in the presence of BamHI enzyme. Chromosomal DNA was prepared from two pools of 50 transformants (lanes 1 and 2) and from one pool of 1000 transformants (lane 3). DNA was prepared as described in Methods and digested with Clal. The fragments were separated by electrophoresis, transferred to a Nytran membrane and probed with a fragment containing the Psrl–Scal fragment encoding \textit{URA3}. The fragment marked ‘1’ corresponds to the chromosomal \textit{URA3} locus.

was digested with Clal and analysed by Southern blot hybridization using the \textit{URA3} gene as the probe. The chromosomal \textit{URA3} locus in all strains contained a fragment that hybridized to the probe (Fig. 2a). This fragment, which is seen when chromosomal DNA from the starting strain SGY-243 is hybridized (data not shown), is prominent in the Southern blot of pools of transformants because each transformant contains this band (Fig. 2b). In addition, each integrated plasmid gave rise to a hybridizing band corresponding to the junction between the transforming plasmid and chromosomal sequence (Fig. 2a). The size of this band is determined by the position of the first Clal site in the chromosomal DNA adjacent to the insertion. In the two pools of 50 transformants, many bands appeared, corresponding to the junctions between plasmid and chromosome in the different transformants (Fig. 2b). The patterns of bands differed between the two pools, indicating that different sequences are adjacent to the transforming plasmids in the strains in the two pools of 50. The blot of the pool of 1000 transformants showed a continuous smear (Fig. 2b), indicating that a large number of different sequences are adjacent to the transforming plasmids in the different strains in the pool. There were no bands that were preferentially represented to a significant extent in the pool of 1000 transformants, demonstrating that there are no preferentially used sites. These results demonstrate that integration at a large number of chromosomal BamHI sites can occur. Therefore, REMI should be useful as a means of mutagenizing the chromosome.

\textit{C. albicans} is a diploid organism (Poulter, 1990) and the insertion mutations generated by REMI would be expected to be heterozygous. Since heterozygous mutants might not exhibit a phenotype, an additional event would be needed to reveal the mutation. For example, if homozygous insertion mutants could be isolated, such mutants could exhibit a phenotypic defect. Alternatively, since a heterozygous insertion mutation contains only one functional copy of the gene, a point mutation in the remaining functional copy could create a mutant strain with an observable phenotype. Thus, in conjunction with either of these processes, REMI insertion mutagenesis can be useful for mutant isolation in \textit{C. albicans}.

Isolation of a mutant containing an insertion in the \textit{SEC14} gene

To isolate \textit{C. albicans} mutants defective in filamentous growth after REMI mutagenesis, pools of transformants were first irradiated with UV light to stimulate mitotic recombination and generate homozygous insertions (Poulter, 1990). Filtration enrichment, described in Methods, was then used to isolate mutant strains that were defective in filamentous growth. Populations of mutagenized cells were cultured under conditions that favour filamentous growth and then filtered. Cells from the filtrate were grown and then refiltered. After four to six rounds of filtration-enrichment, individual cells were plated and single colonies were screened for defects in filamentous growth. One of the mutants identified by this screen was strain IVS9.

Plasmid DNA was recovered from chromosomal DNA isolated from strain IVS9 to identify the gene that had sustained an insertion in this strain. The chromosomal DNA was digested with an enzyme that does not cleave within the transforming plasmid pCK61, so that the recovered DNA included portions of the \textit{C. albicans}
We attempted to construct a SEC14 null mutant to determine whether SEC14 was essential for filamentous growth. Southern blot analysis revealed that strain IVS9 (sec14::pCK61) was heterozygous for the insertion mutation (data not shown). To determine whether the heterozygous mutation would cause a defect in filamentous growth, strain SGY-243 was transformed with pIS2 after linearization with HindIII. Transformants that were due to homologous recombination were obtained and verified by Southern blot analysis. All 10 recombinants analysed exhibited a wild-type phenotype and were proficient in filamentous growth. These results show that the phenotype of strain IVS9 (sec14::pCK61) is not caused by the insertion mutation in SEC14. Although strain IVS9 was not a homozygous insertion mutant, as expected based on the mutant selection strategy, SEC14 was nevertheless of interest because it had been demonstrated that in Yarrowia lipolytica, a sec14 mutant was defective in filamentous growth (Lopez et al., 1994).

Fig. 3. Deduced amino acid sequence of C. albicans Sec14p aligned with the Sec14 gene products from other yeasts. Underlined amino acids indicate residues that are identical in all four sequences. Boxed residues indicate the 105 aa residues identified to use the C. albicans SEC14 gene. The BamHI site that sustained an insertion of pCK61 in strain IVS9 is shown. Ca, C. albicans sequence; accession nos X81937 and U61975 (GenBank); Sc, S. cerevisiae sequence, accession no. X15483 (GenBank); KI, K. lactis sequence, accession no. A37766 (PIR); YL, Y. lipolytica sequence, accession no. L20972 (GenBank). Only the N-terminal 303 aa are shown.

Fig. 4. SEC14 transcripts. (a) Strain CAI-4 was cultured in YPD at room temperature until the culture reached high culture density. The cells were starved in water overnight and then diluted 1/10 or 1/100 into GT2 medium and incubated at room temperature or 40 °C for 4 h. Total RNA was extracted from each culture as described in Methods. RNA was separated by electrophoresis and transferred to Nytran-plus membranes. Blots were probed with probes A, B or C (Fig. 1). Cellular morphologies were determined by examining cells by phase-contrast microscopy. Transcript sizes, determined by comparison with chromosomal DNA by electrophoresis and Northern blot analysis, are shown. (b) Strain CAI-4 was cultured in GT2 medium (yeast cells), probe A; (c) Strain CAI-4 was cultured in GT2 medium (germ tubes), probe A; (d) Strain CAI-4 was cultured in GT2 medium (germ tubes), probe B; (e) Strain CAI-4 was cultured in GT2 medium (yeast cells), probe B; (f) Strain CAI-4 was cultured in GT2 medium (germ tubes), probe B; (g) Strain CAI-4 was cultured in GT2 medium (yeast cells), probe B; (h) Strain CAI-4 was cultured in GT2 medium (germ tubes), probe B.
essential. Although it was possible to construct a sec14+/sec14− heterozygous strain by pop-in/pop-out recombination starting with the wild-type strain CAI-4, it was not possible to construct a sec14+/sec14−::bisG mutant strain starting with the SECl4+/AsecZ4::hisG heterozygous derivative of CAI-4, strain IVS11 (data not shown). These results provide further evidence to support the view that SECl4 is essential in C. albicans.

Two SECl4 transcripts differing at their 3' ends are produced from the SECl4 gene

To investigate the expression of SECl4 during filamentous growth and budding growth, the transcription of SECl4 was studied by Northern blot analysis. Cells of strain CAI-4 were grown at room temperature in YPD to high culture density. The cells were starved in water and then diluted into medium and grown under a variety of conditions. CAI-4 cells grown at room temperature in GT2 medium produced yeast cells and CAI-4 cells grown at 40 °C in GT2 medium produced germ tubes; culture density had no effect on the morphology of the cells.

RNA was extracted from these cells and Northern blots were probed with probe B (Fig. 1), the 953 bp fragment prepared by PCR amplification as described in Methods. As shown in Fig. 4(a), two transcripts of 1600 and 1300 nt were observed in the RNA samples. Quantification of these results showed that in different samples, the relative amounts of the two transcripts were similar. The ratio of upper to lower transcripts was found to be approximately 1:1 for CAI-4 cells producing germ tubes and approximately 1:2 for CAI-4 cells growing as yeast cells at low temperature (Fig. 4a). These ratios were also observed in other experiments using RNA extracted from cells of strain SGY-243 growing as yeast cells or producing germ tubes (data not shown).

Genomic Southern blots were probed to demonstrate that probe B hybridized to only one gene. Probe B hybridized to one SpeI fragment which was cleaved to two smaller fragments by BamHI (Fig. 4b), as expected for the authentic SECl4 gene. As this probe detected two RNA transcripts on the Northern blot (Fig. 4a), the two transcripts were produced from the same gene.

To identify the 5' and 3' ends of the transcripts, SECl4 partial cDNAs were isolated and characterized as described in Methods. One predominant 5' end and one predominant polyadenylation site were identified. At the 5' end, three of four cDNA clones were found to begin at a position corresponding to nt 214 in the genomic sequence of Monteoliva et al. (1996). The fourth clone had a 5' UTR that was 48 nt shorter. At the 3' end, seven of nine cDNA clones were found to contain the identical site of polyadenylation, 149 nt beyond the putative end of the coding sequence (Fig. 5). The remaining two clones had 3' UTRs that were 9 and 14 bases shorter than the other clones, respectively. Although the sequences specifying polyadenylation have not been characterized in C. albicans, sequences matching the efficiency element and positioning element of S. cerevisiae (Guo & Sherman, 1995) were found upstream of the polyadenylation site (Fig. 5).

Thus, from the cDNA analysis only one 5' end and one 3' end were identified. To determine whether the two transcripts differed at their 3' ends, 5' ends or both, hybridization to probes upstream and downstream of the characterized ends was performed. Northern and
that the 1600 nt transcript is a probe A hybridized well in a Southern blot (Fig. 4b), no SEC14 PCR amplification of cDNA was performed to confirm transcript and that the two transcripts do not differ hybridization was seen on the Northern blot (Fig. 4a), conclude that two transcripts are produced from the indicating that the sequences are not present in either end of the gene is contained on this fragment. Although sec14ts SpeI site to the downstream SpeI site complemented a significantly at their ends. Based on these results, we conclude that two transcripts are produced from the SEC14 gene and they differ at their 3' ends. Based on these results, we conclude that two transcripts are produced from the SEC14 gene and they differ at their 3' ends.

To test the 5' end, the blots were probed with probe A (Fig. 1), an approximately 800 bp SpeI–AccI fragment that ends 98 nt upstream of the 5' end characterized above. Monteoliva et al. (1996) demonstrated that a C. albicans SEC14+ clone extending from this upstream SpeI site to the downstream SpeI site complemented a sec4ts mutation in S. cerevisiae, suggesting that the 5' end of the gene is contained on this fragment. Although probe A hybridized well in a Southern blot (Fig. 4b), no hybridization was seen on the Northern blot (Fig. 4a), indicating that the sequences are not present in either transcript and that the two transcripts do not differ significantly at their 5' ends. Based on these results, we conclude that two transcripts are produced from the SEC14 gene and they differ at their 3' ends.

PCR amplification of cDNA was performed to confirm that the 1600 nt transcript is a SEC14 transcript with a longer 3' end. To design primers for PCR, the sequence of chromosomal DNA downstream of the SEC14 ORF was determined (Fig. 5). As shown in Fig. 5, a putative polyadenylation site corresponding to the site mapped above was observed and a second putative site, approximately 300 nt further downstream was observed. Sequences further downstream showed that the Sat3A site used to create probe C is probably located in an intergenic region because the sequence lacks ORFs of significant size (data not shown). PCR amplification was performed as described in Methods, using primer MTTM from the SEC14 coding sequence and primers 1, 2 and 3, illustrated in Fig. 5. Primer 1 would be expected to amplify both transcripts, whereas primers 2 and 3 would only amplify the longer transcript. The results shown in Fig. 6 demonstrate that amplified products of the expected sizes were obtained when the cloned SEC14 locus was amplified. Amplified fragments of the same sizes were obtained when cDNA was used as the template (Fig. 6). Therefore, we conclude that a transcript containing SEC14 coding sequences and extending at least 444 nt beyond the coding sequence is present in the mRNA.

DISCUSSION

The original objective of this study was to use insertion mutagenesis by REMI as a method for identifying genes that are important for filamentous growth. Insertion mutagenesis has the advantage of producing mutations that are physically marked. Thus, it is straightforward to recover the DNA from the site of the insertion for analysis. Insertion by the REMI technique has been shown to occur without preference for insertion sites, demonstrating that it could be used for random mutagenesis. Because all insertion mutations were expected to be heterozygous, UV irradiation was used in this study to induce mitotic recombination and mutants defective in filamentous growth were selected using an enrichment strategy. A heterozygous SEC14 insertion mutant was isolated but the insertion mutation was found not to be responsible for the defect in filamentous growth. Therefore, the phenotype of mutant IVS9 was caused by another mutation and it was not possible to determine the nature of the second mutation. It is possible that a sec14 point mutation arose in the structurally normal copy of SEC14, conferring the selected phenotype. Alternatively, it is possible that the UV irradiation induced a mutation in another gene and this second mutation was responsible for the phenotype of IVS9. To permit the use of REMI as a technique for mutagenesis, it appears that a method allowing selection of homozygous insertions will be necessary.

Nevertheless, SEC14 is an interesting gene because of its role in protein secretion and filamentous growth in other yeasts. In S. cerevisiae, SEC14 is essential for growth and is required for proper function of the protein secretory pathway. Bankaitis and coworkers have demonstrated that Sec14p is localized in the Golgi where it plays a role in modulating lipid biosynthesis and may sense the lipid
composition of the Golgi (McGee et al., 1994). Sec14p modulates the activity of the enzyme choline-phosphate cytidylyltransferase (Skinner et al., 1995). It is likely that Sec14p performs a similar function in the C. albicans Golgi and that protein secretion would become blocked in the absence of SEC14 function.

In contrast, in the dimorphic yeast Y. lipolytica, SEC14 is not an essential gene. Inactivation of Y. lipolytica SEC14 leads to cells with undetectable phosphatidylinositol/phosphatidylcholine transfer activity, yet such cells are viable. Furthermore, the protein secretory pathway functions normally in a sec14 null mutant. The only observable defect caused by the sec14 mutation was a defect in the ability to undergo filamentous growth. One interpretation of these results is that Sec14p participates in a cellular function that is unrelated to the protein secretory pathway during filamentous growth. Alternatively, it is possible that there is another molecule that performs the essential function of Sec14p in the protein secretion pathway in yeast cells but not in hyphae.

Based on these results, it is possible that Sec14p plays two roles in C. albicans. Sec14p may perform an essential function in the protein secretory pathway and a second function that is needed during filamentous growth. The demonstration that two transcripts are produced from SEC14 suggests that the activity of Sec14p could be regulated. The two transcripts may allow Sec14p activity to respond to different environmental conditions, so that Sec14p can perform different functions. In S. cerevisiae, where Sec14p may perform only one major function, transcription of SEC14 was found to be essentially constitutive and was unaffected by temperature shifts or low phosphate conditions (Vahliensieck et al., 1995). In C. albicans, alteration of Sec14p activity could occur in response to environmental conditions as cells prepare for producing germ tubes.

The UTRs of mRNAs affect gene function in a variety of ways. The 3′ sequences affect translational efficiency, message stability and message localization (Decker & Parker, 1995). The 5′ UTRs play a role in translational regulation (Kozak, 1991). In S. cerevisiae, examples of differential polyadenylation have been described. The CBP1 gene, encoding a protein that stabilizes pre-mRNA encoding cytochrome b, utilizes two different polyadenylation sites to produce a long and short transcript (Mayer & Dieckmann, 1989). Only the long transcript encodes the entire Cbp1p coding sequence and this transcript is required for growth of cells by respiration. The levels of the two transcripts are affected by the carbon source used for growth of the cells, providing a mechanism for regulation of Cbp1p production. In the case of SEC14, both transcripts encode the Sec14p coding sequence. However, the two transcripts could differ in their efficiency of translation.

Localization of mRNA transcripts is common in Drosophila development and contributes to the establishment of embryonic polarity (St Johnston, 1995). For example, the bicoid mRNA is localized to the anterior pole of the oocyte. Upon translation, the bicoid gene product establishes a gradient that contributes to the definition of the head, thorax and abdomen of the embryo. Sequences in the 3′ end have been shown to be responsible for mRNA localization (Macdonald & Struhl, 1988). It is possible that the different 3′ ends of SEC14 may lead to localization of the mRNA in different locations in the cell.

Alternatively, the 3′ ends of C. albicans SEC14 transcripts may permit efficient expression under different conditions of growth. In the mouse, alternative splicing in the 5′ UTR of HSP47 permits efficient translation under heat shock conditions (Takechi et al., 1994).

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REFERENCES


Expression of the C. albicans SEC14 gene


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