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Cloning and Characterization of *ECE1*, a Gene Expressed in Association with Cell Elongation of the Dimorphic Pathogen *Candida albicans*

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The gene *ECE1* (extent of cell elongation 1) was isolated by differential hybridization screening of a *Candida albicans* cDNA library by using probes derived from populations of yeast cells or hyphae. Expression of this gene was not detected when *C. albicans* grew as a budding yeast cell but was observed within 30 min after cells had been induced to form hyphae. In all strains tested, regardless of the induction signal, *ECE1* expression correlated with the extent of cell elongation. The genomic version of *ECE1* was cloned and sequenced. The deduced 271-amino-acid polypeptide consisted of eight tandem repeats of a degenerate 34-amino-acid sequence which contained no discernible homology with other known sequences. An *ECE1* null mutant displayed no morphological alterations, which demonstrated that *ECE1* is not essential for cell elongation or hypha formation despite the strict morphological association of its expression.

Candida albicans can reversibly alter its mode of growth from a budding yeast cell to an elongating hypha, depending upon its environment. This dimorphic property is believed to be involved in the pathogenicity of this fungus. Although the hyphal form has been shown to adhere more readily than the budding form to human epithelial cells (22), the morphological flexibility this pathogen gains from switching between the yeast and hyphal forms may also be important in allowing *C. albicans* to penetrate and proliferate in a wide variety of host tissues (30). Despite the significance of dimorphism to the biology of *C. albicans*, the regulation of this process is not understood.

On the basis of the assumption that differential expression is responsible, in part, for the dimorphic process, biochemical and immunological approaches have been applied to the identification of form-specific molecules that might be involved. Differences have been demonstrated by polyacrylamide gel electrophoresis of protein extracts prepared from *C. albicans* grown in either the yeast or hyphal form. One investigation revealed five polypeptides which were apparently specific to budding cells (6). In another study, three polypeptides were found to be exclusively expressed in yeast cells cultured at 25°C while four proteins were specific to hyphae cultured at 37°C (1). In neither of these studies, however, were the polypeptides shown to be form specific rather than temperature specific. By utilizing pH to modulate morphology, only a single polypeptide of the 374 examined was found to be specific to yeast cells and another was unique to hyphal cells (13). The identities and functions of these form-specific proteins are unknown.

Several groups have demonstrated antigenic differences between yeast cells and hyphae (9, 35, 45, 47), despite strain variations (36) and the dynamic nature of the cell wall (5, 10). However, the characterization of these antigens and their role in dimorphism has been limited.

Genetic approaches have thus far provided limited insight into the dimorphism of *C. albicans*. Several groups have reported the isolation of mutants which persist in either the yeast (8a) or hyphal (16, 20) form. Analysis of these mutants is hampered by the difficulties inherent in the genetic manipulation of an asexual diploid organism such as *C. albicans*. It is not known whether the morphological phenotypes of these mutants is a result of mutations at single or multiple loci since the lack of a sexual cycle precludes segregation analysis. However, parasexual techniques have permitted complementation studies which suggested that multiple loci are involved in dimorphism (16). The genes affected in these mutants and their role in dimorphism have yet to be defined.

The development of genetic transformation (23) and gene disruption techniques (21) applicable to *C. albicans* provided the requisite tools for a molecular genetic approach to the analysis of dimorphism. Toward this end, we have used differential hybridization screening to isolate genes which exhibited morphology-dependent expression. In this report we describe the characterization of the *ECE1* gene (extent of cell elongation). *ECE1* was expressed in association with hypha formation and was one of the most abundantly expressed genes in these cells.

MATERIALS AND METHODS

***C. albicans* strains.** *C. albicans* SC5314 (23), used in the construction of cDNA and genomic libraries, and strain SGY243 (21) (*ade2/ade2 Δura3::ADE2/Δura3::ADE2*) were provided by the Squibb Institute for Medical Research. The clinical isolates ATCC 38696 and 3153A were obtained from Paula Sundstrom and David Soll, respectively.

Strain CAI4 (*Δura3::λimm434/Δura3::λimm434*) is a Ura$^-$ derivative of strain SC5314 (14). Strains CAF5-1 (*Δura3::λimm434/Δura3::λimm434 Δece1::hisG::I-SceI-URA3-hisG::I-SceI/ECE1*) and CAF5-2 (*Δura3::λimm434/Δura3::λimm434 ECE1/Δece1::hisG::I-SceI-URA3-hisG::I-SceI*) are heterozygous for a deletion of alternate alleles in the *ECE1* gene (14). Strain CAF6-8 (*Δura3::λimm434/Δura3::λimm434 ece1::*

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hisG::I-SceI/Δece1::hisG::I-SceI-URA3-hisG::I-SceI) is homozygous for the *ECE1* deletion (14).

Growth and hyphal induction. *C. albicans* was routinely propagated in YEPD (43) at 30°C. The clinical isolates, strains SC5314, ATCC 38696, and 3153A, were induced to form germ tubes either in a synthetic amino-acid-rich medium (27) or in imidazole buffer (pH 7.0) with the addition of a specific inducer (42).

Yeast cells were inoculated into the medium of Lee et al. (27) adjusted to pH 4.5 and shaken at 180 rpm at 25°C until the culture had reached the late logarithmic or early stationary growth phase. Hyphae were formed after transfer of the cells to fresh medium (pH 6.5) and incubation at 37°C. The final cell density was 8×10^6 cells per ml. Alternatively, yeast cells were cultured in defined minimal medium (41) and induced to form hyphae as described by Shepherd et al. (42). Hyphae were induced at 37°C in 10 mM imidazole-HCl (pH 7.0) in the presence of 2.5 mM *N*-acetylglucosamine (42), 4% serum (42), or 10 mM L-proline (11).

The auxotrophic strain SGY423, which has been heavily mutagenized (22, 25), failed to undergo filamentation in the medium of Lee et al. (27) but did form hyphae in tissue culture medium TCM199 (GIBCO-BRL) adjusted to pH 7.0 with 7.5% $NaHCO_3$. After incubation at 25°C for 6 days on YEPD plates supplemented with uridine (25 μg/ml), one colony of SGY243, containing approximately 10^8 cells, was transferred to TCM199 (100 ml) supplemented with uridine (25 μg/ml) and serum (4%) and shaken at 100 rpm at 37°C.

Cell morphology was monitored by light microscopy and photographed with an Olympus C-35 inverted camera.

Isolation of nucleic acids. Genomic DNA was extracted from *C. albicans* by the method of Scherer and Stevens (40). Total RNA was obtained by the method of Langford and Gallwitz (26) with the modification that when serum was employed to induce hyphal formation, the cells were washed two additional times in the presence of 0.5% sodium dodecyl sulfate to remove serum proteins prior to phenol extraction.

***C. albicans* cDNA and genomic library construction.** The cDNA library was prepared from RNA of *C. albicans* SC5314 (46). Cells were grown to the stationary phase in YEPD at 30°C, and germ tubes were then induced by transfer of the yeast cells to TCM199. After 60 min of incubation at 37°C, the cells were harvested and poly(A)$^+$ mRNA was isolated (3). This RNA was used by Stratagene laboratories to construct the cDNA library in the vector Lambda Zap II. The library contained approximately 2×10^6 primary PFU with about 5% nonrecombinants.

The genomic lambda library was prepared in λGEM12 (Promega). Genomic DNA was isolated from strain SC5314, partially digested with *Sau*3A, and ligated into the *Xba*I site of the vector λGEM12 following a partial fill-in reaction as described by the manufacturer. The library was initially plated on *Escherichia coli* KW251 and subsequently plated on strain LE392. This library was designated CA5314-GEM12 (50). Hybridization screening of the lambda libraries was conducted as described by Carlock (8b).

Southern and Northern (RNA) blot analysis. Standard DNA electrophoretic techniques and formaldehyde RNA gels were employed (38). Blotting was carried out with Hybond-N nylon membranes (Amersham) as described in the manufacturer's instructions. DNA fragments used as hybridization probes were isolated from agarose gels by using Gene Clean (Bio 101, Inc.) and were labelled by employing random oligonucleotides as primers as described by the manufacturer (U.S. Biochemicals). DNA containing the *C. albicans* actin gene was used as a control in Northern blot hybridizations and was kindly provided by P. Sundstrom.

Sequencing. Appropriate DNA fragments from genomic clone pECE041 and cDNA clones pECEC01 and pECEC41 were subcloned into pBluescript KS$^{+/-}$ (Stratagene). The DNA sequence was determined (39) from single-stranded sequencing templates by using Sequenase T7 polymerase (U.S. Biochemicals). Nucleotide and protein sequence analyses were performed with the Wisconsin Genetics Computer Group sequence analysis software package, version 7.0 (12). Homology searches of the GenBank data base were conducted with the FASTA program of Pearson and Lipman (32).

Transformation of *C. albicans*. *C. albicans* spheroplasts were transformed by using the *Saccharomyces cerevisiae* protocol (23, 43). Buffering the transforming DNA with 50 mM buffer (pH 7.4) prior to mixing it with spheroplasts significantly increased the number of transformants obtained (data not shown).

Nucleotide sequence accession number. The nucleotide sequence of the *ECE1* gene has been deposited in the GenBank data base under accession number L17087.

RESULTS

Differential screen of *C. albicans* cDNA library. A differential screen of the *C. albicans* cDNA library was conducted by using first-strand cDNA probes prepared from the RNA of cells exhibiting a yeast or hyphal morphology. To prepare the probes, total RNA was extracted from cells of strain SC5314 following a 90-min incubation in the medium of Lee et al. (27) under conditions which lead to exhibition of the yeast (pH 4.5, 25°C) or hyphal (pH 6.5, 37°C) morphology. Two enrichment steps for the selection of poly(A)$^+$ RNA using oligo(dT)-cellulose chromatography (3) were undertaken, and ^{32}P-labelled first-strand cDNA was then synthesized with oligo(dT) as a primer (7, 37). After first-strand cDNA synthesis, the RNA was hydrolyzed with 5 N NaOH.

Approximately 2,000 phages from the cDNA library were plated, and duplicate plaque lifts of each plate were prepared. One of each duplicate filter was hybridized with either the yeast-cell-derived or hypha-derived probe. In this manner, we identified 67 recombinant phages which exhibited a more intense signal when hybridized with the first-strand cDNA derived from hyphae than when hybridized with the yeast-cell-derived probe. Only a single phage was observed to give the converse results.

Multiple representatives of particular genes within this group of 67 hypha-specific clones were revealed by cross-hybridization of individual phage with a panel of the 67 differentially expressed cDNA clones. Eleven clones were found to be unique among the group, and the remaining three were represented by 8, 20, and 27 clones.

Expression of *ECE1* correlates with cell elongation. One of the multiply represented clones, pECEC01, appeared to be highly transcribed when *C. albicans* grew as hyphae, being represented by 27 of a total of approximately 2,000 cDNA-containing phage. The results of Northern blot hybridizations were in accord with this conclusion. By using the 0.8-kb insert from pECEC01 as a hybridization probe, an abundant transcript of approximately 1 kb was detected in RNA samples from cells of strain SC5314 induced to form hyphae in the medium of Lee et al. (27) (Fig. 1, lane 4). However, no transcript was detected in RNA samples from strain SC5314 growing as yeast cells, even with prolonged exposure of the blot (Fig. 1, lane 3). Such a high level of

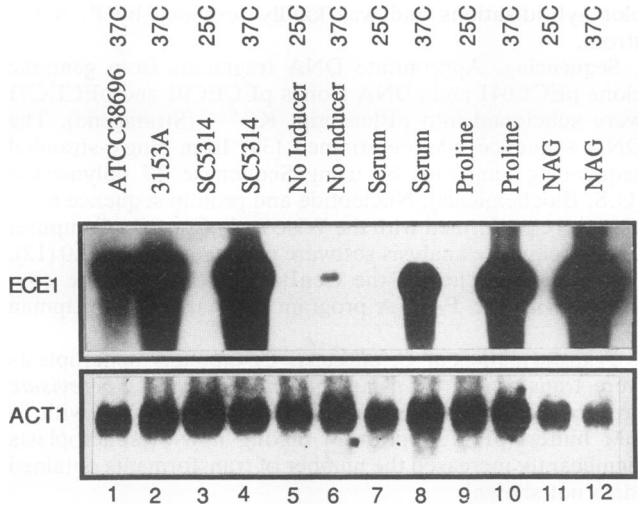


FIG. 1. Northern blot analysis of *ECE1* expression in various strains and under various culture conditions. Clinical isolates ATCC 38696 and 3153A were induced to form hyphae in the medium of Lee et al. (27) at pH 6.5 and 37°C (lanes 1 and 2). Strain SC5314 was grown in the same medium as yeast cells (pH 4.5, 25°C; lane 3) and as hyphae (pH 6.5, 37°C; lane 4). Strain SC5314 was also incubated in 10 mM imidazole buffer (pH 7.0) at either 25 or 37°C in the presence of no inducer (lanes 5 and 6), serum (lanes 7 and 8), L-proline (lanes 9 and 10), or *N*-acetylglucosamine (NAG; lanes 11 and 12). After 2 h of incubation, the cultures were harvested and the total RNA was extracted. Twenty micrograms of each sample was analyzed by Northern blot hybridization, *ECE1* expression being detected by using the *ECE1* cDNA clone pECEC41 as a hybridization probe (upper panel). Control hybridizations with the *C. albicans* actin gene (ACT1) demonstrated equal loading in each lane (lower panel).

expression suggested that the *ECE1* gene product may be important in the dimorphic process, and hence, it was chosen for further study.

Having performed the differential screen with RNA extracted from strain SC5314 grown in the medium of Lee et al. (27), we wanted to test whether *ECE1* expression was actually associated with the morphological transformation itself or whether its expression was specific to either strain SC5314 or the induction medium. We did this by examining *ECE1* expression in other clinical isolates of *C. albicans* and by employing different conditions to induce hypha formation.

Northern blot analysis demonstrated that the *ECE1* transcript was expressed when two different strains, ATCC 38696 and 3153A, were induced to form hyphae in the medium of Lee et al. (27) (Fig. 1, lanes 1 and 2). It can also be seen in Fig. 1 that when hyphae are induced at 37°C with serum (lane 8), L-proline (lane 10), or *N*-acetylglucosamine (lane 12), *ECE1* was highly expressed. In the corresponding control cultures incubated at 25°C, no *ECE1* transcript was detected (Fig. 1, lanes 7, 9, and 11). A low level of *ECE1* expression was detected in cells incubated at 37°C, even when no inducer was added to the medium, but a small proportion of the population, approximately 1%, was observed to produce germ tubes (Fig. 1, lane 6). These results indicated that *ECE1* expression was not peculiar to strain SC5314, nor to the induction medium, but rather that *ECE1* is expressed whenever true hyphae are formed.

To understand more about how the environment was contributing to *ECE1* induction, strain SC5314 was grown in the medium of Lee et al. (27) adjusted to either pH 4.5 or 6.5 and incubated at 25 or 37°C. The extent of *ECE1* expression was examined by Northern blot analysis. The morphological status of SC5314 when grown under these various conditions is shown in Fig. 2a. As can be seen from these photomicrographs, at pH 4.5 and 25°C, SC5314 grew entirely by budding, with all of the cells having an ovoid shape (Fig. 2aA). However, at pH 6.5 and 37°C, almost all of the cells grew as hyphae (Fig. 2aD). If either the pH or the temperature was suboptimal for true hyphal formation, an intermediate pseudohyphal morphology was observed. When the temperature was 25°C and the pH was 6.5, the cells closely resembled the yeast form but were slightly elongated (Fig. 2aB). When the temperature was 37°C and the pH was 4.5, most of the cells were considerably elongated but did not form true hyphae (Fig. 2aC).

Northern blot analysis of RNA from cells grown under these various conditions demonstrated that both pH and temperature were contributing to *ECE1* expression but that neither was sufficient in itself to induce maximal expression of *ECE1*. Instead, there appeared to be a graded increase in *ECE1* expression from undetectable levels in cells cultured at pH 4.5 and 25°C (Fig. 2b, lane 1) to a low level of expression in cells cultured at pH 6.5 and 25°C (Fig. 2b, lane 2), with increased expression in cells cultured at pH 4.5 and 37°C (Fig. 2b, lane 3) and a maximal level of expression in cells cultured at pH 6.5 and 37°C (Fig. 2b, lane 4). From these results, it appears that *ECE1* expression correlated with cell elongation, i.e., the more elongated the cells, the greater the level of *ECE1* expression. Hence, the corresponding gene was designated *ECE1* (extent of cell elongation).

It was important at this stage to demonstrate that *ECE1* expression was associated with cell morphology rather than being due to the pH and temperature conditions which were being employed to induce cell elongation. To discriminate between these two possibilities, we employed strain SGY243, a uridine auxotroph, which does not form hyphae in the uridine-supplemented medium of Lee et al. (27) at pH 6.5 and 37°C (data not shown). By using this strain, we could therefore distinguish whether *ECE1* expression was the result of a combination of elevated pH and temperature or whether it was truly related to cell elongation. Accordingly, RNA was extracted from cells of strain SGY243 grown in the uridine-supplemented medium of Lee et al. (27) adjusted to pH 6.5 and incubated at 37°C. In addition, RNA was prepared from cells incubated in TCM199 at 37°C, conditions which induce hypha formation by strain SGY243 (data not shown). As can be seen in Fig. 3, *ECE1* expression coincided with cell elongation (Fig. 3, lane 4) and was not induced in response to the culture conditions per se (Fig. 3, lane 2).

To investigate further the association between *ECE1* activation and hyphal elongation, we undertook a temporal analysis of *ECE1* expression following exposure of the cells to the induction signal. It is clear from the Northern blot shown in Fig. 4 that *ECE1* expression is rapidly induced, being detectable within 30 min of stimulating the cells to form hyphae (Fig. 4, lane 2). This early activation of *ECE1* expression suggested that *ECE1* may actually be involved in the process of cell extension, the first morphological sign of which is the emergence of a germ tube approximately 90 min after induction, rather than being expressed as a consequence of morphological differentiation.

In conclusion, the results demonstrated that the expression of *ECE1* correlates with cell extension. *ECE1* was highly expressed when hyphae were formed, regardless of

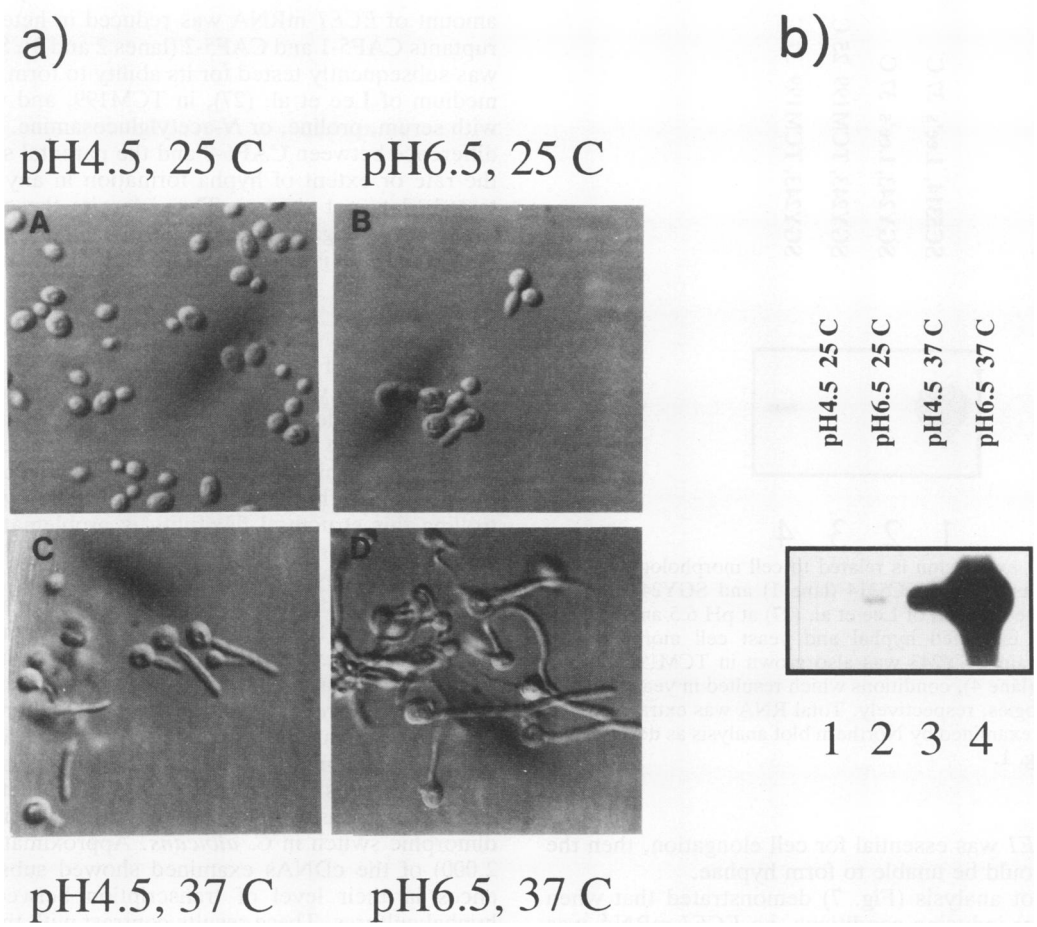


FIG. 2. Comparison of cell morphology and *ECE1* expression. (a) Photomicrographs showing the morphology of strain SC5314 incubated in the medium of Lee et al. (27) at various temperatures and pH. The temperature and pH of the medium are indicated for each panel. (b) Northern blot analysis of the effect of culture temperature and pH on the expression of *ECE1*. Total RNA was prepared from each of the cultures shown in panel a and examined by Northern blot hybridization as described in the legend to Fig. 1.

the induction signal, and *ECE1* expression occurred soon after the stimulus to form hyphae was given.

Nucleotide and deduced amino acid sequence of *ECE1* gene. From the pattern of *ECE1* expression detailed above, it appeared that *ECE1* may play a role in the process of hyphal formation. To investigate the putative role of *ECE1* in dimorphism, the nucleotide sequence of the gene was determined with the aim of comparing the structure of the presumptive *ECE1* gene product with proteins of known function.

A genomic clone of the gene was obtained by hybridization screening of the genomic lambda library CA5314-GEM12 by using the insert from the *ECE1* cDNA clone pECEC01. Subsequently, a 4.2-kb *Bam*HI fragment was subcloned from the insert of one of the pECEC01-hybridizing genomic lambda clones (Fig. 5a). The pECEC01-hybridizing sequences within the 4.2-kb *Bam*HI fragment were localized by Southern blot analysis of restriction enzyme digests, and the nucleotide sequence of a 1,710-bp region containing the hybridizing sequences was determined (Fig. 5b).

The nucleotide sequence contained two overlapping open reading frames (ORF). The longer ORF extends from the ATG start codon at nucleotide 711, while the shorter ORF starts from the ATG at position 795. Both ORFs terminate at the stop codon TAA at nucleotide 1524. We have not determined which of these two in-frame AUG codons acts as the site for initiation of translation. In common with other *C. albicans* genes, the presumptive *ECE1* promoter is highly AT rich and no consensus splice signals characteristic of introns were found within the presumptive coding region (24). Polyadenylation of the *ECE1* transcript occurs at positions 1709 or 1710, as determined by sequence analysis of two *ECE1* cDNA clones (data not shown).

The longer ORF would encode a polypeptide of 271 amino acid residues, having a predicted molecular size of 28,886 Da and a pI of 5.51. Interestingly, this protein consists of eight degenerate repeats, 34 amino acids in length, as shown in Fig. 6. An internal proline (P) residue and terminal lysine (K), arginine (R), and aspartic acid (D) residues are highly conserved within this repeat, perhaps reflecting a functional role of these residues. No obvious homology was found when this sequence was compared with other sequences in the GenBank and EMBL data bases nor was any functional motif identified when the protein was compared with sequences in the Prosite data base.

Phenotype of *ECE1* null mutant. To determine whether *ECE1* expression was necessary for morphogenesis, an *ECE1* null mutant (14) was examined for its ability to form

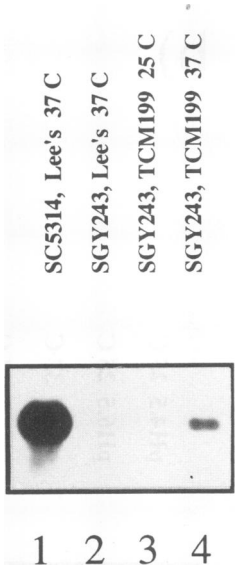


FIG. 3. *ECE1* expression is related to cell morphology and not culture conditions. Strains SC5314 (lane 1) and SGY243 (lane 2) were grown in the medium of Lee et al. (27) at pH 6.5 and 37°C, in which the cells exhibited hyphal and yeast cell morphologies, respectively. Strain SGY243 was also grown in TCM199 at 25°C (lane 3) or 37°C (lane 4), conditions which resulted in yeast cell and hyphal morphologies, respectively. Total RNA was extracted from the cultures and examined by Northern blot analysis as described in the legend to Fig. 1.

hyphae. If *ECE1* was essential for cell elongation, then the null mutant should be unable to form hyphae.

Northern blot analysis (Fig. 7) demonstrated that when incubated under inducing conditions, no *ECE1* mRNA was present in the null mutant CAF6-8 (lane 4) and that the amount of *ECE1* mRNA was reduced in heterozygous disruptants CAF5-1 and CAF5-2 (lanes 2 and 3). Strain CAF6-8 was subsequently tested for its ability to form hyphae in the medium of Lee et al. (27), in TCM199, and when induced with serum, proline, or *N*-acetylglucosamine. There was no difference between CAF6-8 and the parental strain in either the rate or extent of hypha formation in any of the media tested (data not shown). Thus, despite the correlation between cell elongation and *ECE1* expression, the product of the *ECE1* gene is not required for hypha formation.

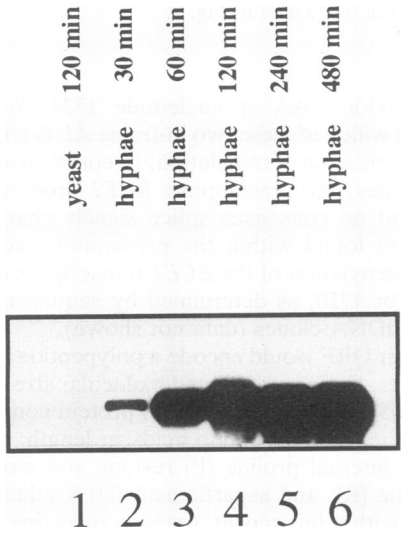


FIG. 4. Temporal expression of *ECE1* during germ tube emergence. RNA was extracted from cultures of strain SC5314 grown as yeast cells in the medium of Lee et al. (27) at pH 4.5 and 25°C (lane 1) and from cells incubated in the same medium at pH 6.5 and 37°C for 30 (lane 2), 60 (lane 3), 120 (lane 4), 180 (lane 5) and 240 (lane 6) min, conditions which stimulated hyphal outgrowth. *ECE1* expression was analyzed by Northern blot hybridization as described in the legend to Fig. 1.

DISCUSSION

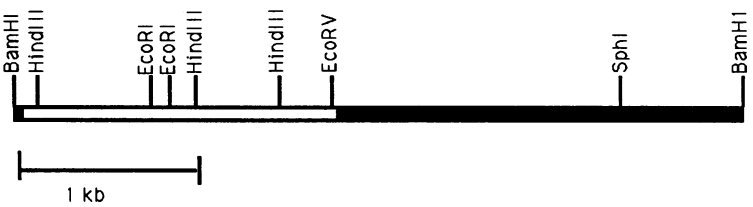
The ability of *C. albicans* to switch its mode of growth from a budding yeast cell to an elongating hypha is believed to confer considerable advantage of this asexual fungus in its ability to invade a wide variety of tissues and evade the host defense mechanisms. Pursuing a classical genetic approach to understand the molecular mechanisms involved in controlling this structural flexibility is problematic because of the diploid nature of this pathogenic fungus (31, 49) and the failure to identify a sexual cycle. As such, we have adopted a molecular genetic approach, the differential screen of a cDNA library, to identify genes whose expression varies when *C. albicans* grows by budding or by hyphal elongation.

This type of approach has been used to demonstrate the differential expression of numerous genes during the development of various phylogenetically lower eukaryotes including *Aspergillus nidulans* (19, 48) and *Neurospora crassa* (4). By utilizing this method, we have shown that considerable changes in transcriptional activity are associated with the dimorphic switch in *C. albicans*. Approximately 3% (68 of 2,000) of the cDNAs examined showed substantial differences in their level of transcription between yeast and hyphal cultures. These results contrast with the conclusions drawn from previous biochemical studies which led to a hypothesis that lowered the importance of differential gene expression and promoted the involvement of subtle temporal and spatial differences (30, 44). Although the present study demonstrated significant differences in the levels of expression of several genes, it remains unclear whether these changes are involved in morphogenesis or whether they are simply associated with the process.

The gene *ECE1* was picked for further study because it was very highly expressed and its expression correlated with cell elongation. The abundance of *ECE1* mRNA was apparent from the differential screen where this gene was represented by 27 of 2,000 cDNA clones (1%), and it is clear from the temporal analysis that expression increases from this 2-h level as elongation continues. Of particular interest was the finding that *ECE1* exhibited incremental expression in pseudohyphal stages of development. This suggests that the pseudohyphal morphology may reflect an intermediate structure produced as the result of a graded expression of the constituents of budding yeast cells and elongating hyphae rather than constituting a distinct morphological form (29).

While no similarities were found between the putative *ECE1* protein and those present in the GenBank data base, the sequence analysis revealed that the *ECE1* polypeptide did have an unusual primary structure, being composed of a degenerate 34-amino-acid repeat tandemly repeated eight times. Interestingly, several other proteins have been identified, primarily from yeasts and fungi, which contain a multiply repeated 34-amino-acid motif named the tetratricopeptide. However, *ECE1* does not possess homology with the conserved residues within this repeated motif (17).

a)



b)

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1
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81
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641
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711/1
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Met lys phe ser lys ile ala cys ala thr val phe ala leu ser ser gln ala ala ile
771/21
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ile his his ala pro glu phe asn met lys arg asp val ala pro ala ala pro ala ala
831/41
CCA GCT GAC CAA GCA CCT ACT GTT CCT GCA CCT CAA GAA TTC AAT ACT GCT ATT ACC AAA
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891/61
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arg ser ile ile gly ile ile met gly ile leu gly asn ile pro gln val ile gln ile
951/81
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ile met ser ile val lys ala phe lys gly asn lys arg glu asp ile asp ser val val
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1071/121
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val ala ser thr lys arg asp gly ala asn asp asp val ala asn ala val val arg leu
1131/141
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pro glu ile val ala arg val ala thr gly val gln gln ser ile glu asn ala lys arg
1191/161
GAT GGC GTT CCA GAT GTT GGC CTT AAT CTT GTT GCT AAT GCT CCA AGA CTT ATC TCT AAC
asp gly val pro asp val gly leu asn leu val ala asn ala pro arg leu ile ser asn
1251/181
GTT TTT GAT GGC GTC CTG GAA ACT GTT CAA CAA GCT AAG AGA GAT GGT CTT GAA GAT TTT
val phe asp gly val leu glu thr val gln gln ala lys arg asp gly leu glu asp phe
1311/201
CTT GAT GAA CTT CTT CAA AGA CTC CCA CAA CTC ATT ACT AGA TCA GCT GAA TCT GCT TTG
leu asp glu leu leu gln arg leu pro gln leu ile thr arg ser ala glu ser ala leu
1371/221
AAA GAC AGT CAA CCA GTT AAA AGA GAT GCC GGC TCA GTA GCA CTT AGC AAT TTA ATC AAA
lys asp ser gln pro val lys arg asp ala gly ser val ala leu ser asn leu ile lys
1431/241
AAG AGC ATT GAA ACT GTC GGT ATT GAA AAT GCT GCT CAA ATT GTT TCA GAA AGA GAT ATT
lys ser ile glu thr val gly ile glu asn ala ala gln ile val ser glu arg asp ile
1491/261
TCT TCT TTG ATT GAA GAA TAT TTC GGA AAA GCT TAA
ser ser leu ile glu glu tyr phe gly lys ala OCH

1527
ATGCTCAGCAGATAAAAATTTGTTTTCCACAAGCTTAATCTTTTATTCCATAAGTCTTGTGACACTTTTGCTGTAACTGA
1607
TTTTTTTAATTGTTGTTTTTTGTGGTGGTGTTGAATTGCTTTAATATCATTTTGATAGCATTCACACCATAGTTTATATT
1687
ACCTATATTTTACTTAGATATCAA
```

FIG. 5. Structure of the *ECE1* gene. (a) Restriction map of the 4.2-kb *Bam*HI genomic DNA fragment containing the *ECE1* gene. The open box shows the location of the sequenced region. (b) Nucleotide and deduced amino acid sequences of *ECE1*.

ECE1 is highly expressed in a tightly regulated fashion, but its expression is not required for growth or hyphal development of *C. albicans* under the conditions employed in the laboratory. It would seem surprising that *C. albicans* would expend the considerable energy involved in expressing *ECE1* if it provided no biological advantage; indeed, evolutionary pressures might have been expected to have eliminated it from the genome if this was indeed the case. However, other examples exist where fungal genes specifically expressed during differentiation have been shown to be dispensable for growth and development. The 38-kb SpoC1 cluster in the filamentous fungus *A. nidulans* contains 14 genes which together make up approximately 2% of the total mRNA produced during asexual spore production; this region can be eliminated without any detectable phenotype (2). Similarly, various sporulation-specific genes from *Sac-*

```
  1 MKFSKIACATVFALSSQAAIIHHAPEFNM..KRD
 33 VAPAAPAAPADQAPTVPAPQEFNTAI....TKR.
 62 SIIGIIMGILGNIPQVI.QIIMSIVKAFKGNKRE
 95 DIDSVVAGIIADMPFVVRAVDTAMT.SVASTKRD
128 GANDDVANAVVRLPEIVARVATGVQQSIENAKRD
162 GVPDVGLNLVANAPRLISNVFDGVLETVQQAKRD
196 GLEDFLDELLQRLPQLITRSAESALKDSQPVKRD
230 AGSVALSNLIKKSIETVGIENAAQIVSE...RD
260 ISSLIEEYFGKA
```

FIG. 6. Alignment of the 34-amino-acid repeat within the putative *ECE1* protein. Boxes indicate highly conserved residues.

charomyces cerevisiae have been deleted, again with no discernible changes (15, 18, 28, 33, 34). While no homologs of *ECE1* have been detected, it is conceivable that the *ECE1* function is redundant. Another possibility is that *ECE1* functions during cell adhesion, cell invasion, cell metabolism, or perhaps in the evasion of the host defense mechanisms when *C. albicans* grows in its natural environment.

In spite of the lack of a discernible phenotype associated with the *ECE1* null mutant, the data reported here are significant in that they provide the first evidence indicating that germ tube formation in *C. albicans* entails differential expression of a number of genes. Furthermore, the coordinate regulation of genes such as *ECE1*, which are not directly involved in morphogenesis, indicates that morphogenesis of *C. albicans* is more than a change in cell shape and entails the programmed expression of many other functions which may account for the unique biochemical and physiological properties of the hyphal form of the organism.

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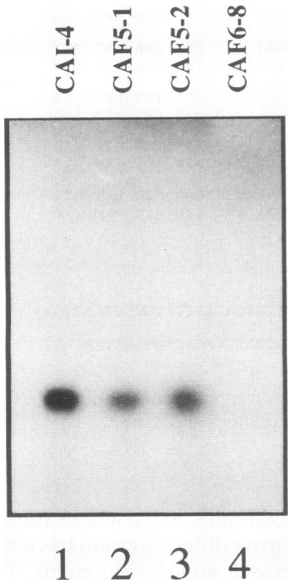

FIG. 7. *ECE1* expression in heterozygous and homozygous deletion mutants. The indicated strains were induced in the medium of Lee et al. (27) at pH 6.5 and 37°C for 90 min. Total RNA was extracted and subjected to Northern blot analysis as described in the legend to Fig. 1.

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