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Dosage-dependent functions of fatty acid desaturase Ole1p in growth and morphogenesis of *Candida albicans*

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Conditions in the infected human host trigger virulence attributes of the fungal pathogen *Candida albicans*. Specific inducers and elevated temperatures lead to hyphal development or regulate chlamydospore development. To explore if these processes are affected by membrane lipids, an investigation of the functions of the Ole1 fatty acid desaturase (stearoyl-CoA desaturase) in *C. albicans*, which synthesizes oleic acid, was undertaken. A conditional strain expressing *OLE1* from the regulatable *MET3* promoter was unable to grow in repressing conditions, indicating that *OLE1* is an essential gene. In contrast, a mutant lacking both alleles of *OLE2*, encoding a Ole1p homologue, was viable and had no apparent phenotypes. Partial repression of *MET3p–OLE1* slightly lowered oleic acid levels and decreased membrane fluidity; these conditions permitted growth in the yeast form, but prevented hyphal development in aerobic conditions and blocked the formation of chlamydospores. In contrast, in hypoxic conditions, which trigger an alternative morphogenetic pathway, hyphal morphogenesis was unaffected. Because aerobic morphogenetic signalling and oleic acid biosynthesis require oxygen, it is proposed that oleic acid may function as a sensor activating specific morphogenetic pathways in normoxic conditions.

INTRODUCTION

The fluidity of cellular membranes is determined to a large extent by their lipid composition and by ambient temperatures. High levels of unsaturated fatty acids, low amounts of sterols in eukaryotic membranes and high temperatures increase fluidity (Carratu *et al.*, 1996; Chatterjee *et al.*, 1997; Horvath *et al.*, 1998). It has been proposed that changes in the physical state of membranes are directly sensed and transmitted by specific signalling pathways triggering protective stress responses (Moskvina *et al.*, 1999). During a heat shock such membrane-induced events could contribute to the complete set of stress responses, which alternatively are activated by protein unfolding (Ananthan *et al.*, 1986; Torok *et al.*, 1997). In yeast an increase in levels of saturated fatty acids within membrane lipids lowered the response to heat shock (Carratu *et al.*, 1996). Similarly, artificially increasing or lowering membrane fluidity in cyanobacteria lowered and, respectively, increased the set point of the heat-shock response (Horvath *et al.*, 1998; Wada *et al.*, 1990; Vigh *et al.*, 1993). Several organisms adapt to low temperatures by increasing the degree of fatty acid desaturation, which leads to an increase in membrane fluidity and simultaneously increases responses to elevated temperatures (Cossins, 1994; Vigh *et al.*, 1998).

In the yeast *Saccharomyces cerevisiae* the *OLE1* gene product encodes a stearoyl-CoA desaturase (EC 1.14.99.5) located in membranes of the endoplasmic reticulum (ER), which transforms the CoA derivatives of palmitic and stearic acid into the corresponding Δ9 monounsaturated palmitoleic ($C_{16:1}$) and oleic acid ($C_{18:1}$) derivatives, respectively (Stukey *et al.*, 1989). The $C_{16:1}$ derivative is the predominant unsaturated fatty acid in *S. cerevisiae*, while other fungi including *Candida* species also produce $C_{18:2}$ (linoleic) and $C_{18:3}$ (linolenic) fatty acids, which are not present in *S. cerevisiae* (reviewed by Mishra *et al.*, 1992). Fatty acid desaturases use electrons in cytochrome b_5 (derived from NADH by NADH-dependent cytochrome b_5 reductase) and molecular oxygen to form a double bond between C-atoms 9 and 10 of fatty acids. Remarkably, fungal fatty acid desaturases contain a cytochrome b_5 domain as integral parts of their enzyme structures, while vertebrate enzymes use separate cytochrome b_5 molecules (Mitchell & Martin, 1995). The active site in the native protein comprises three histidine-rich sequences, which fold to form two iron-binding sites. Yeast Ole1p contains four putative transmembrane regions, which by forming two pairs of membrane-traversing regions could attach the desaturase to the ER membrane and leave most of the

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Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; ER, endoplasmic reticulum.

Ole1p sequences within the cytoplasm (Stukey *et al.*, 1990). *OLE1* expression is regulated by fatty acids, oxygen and temperature. Saturated fatty acids induce a 1·6-fold increase in transcription, while unsaturated fatty acids repress *OLE1* transcription up to 60-fold (McDonough *et al.*, 1992; Bossie & Martin, 1989). At low temperatures and during oxygen limitation, *OLE1* expression is induced (Kwast *et al.*, 1998; Nakagawa *et al.*, 2002). A deletion analysis of the *OLE1* promoter identified a 111 bp fatty acid-regulated region (FAR) which is essential for transcription activation and repression by unsaturated fatty acids (Choi *et al.*, 1996). In addition, the low oxygen response promoter element (LORE) mediates oxygen repression of *OLE1* (Nakagawa *et al.*, 2001; Vasconcelles *et al.*, 2001). Two genes encoding components of fatty acid transporters, *FAA1* and *FAA4*, were found to be essential for unsaturated fatty acid-repression of *OLE1* via FAR sequences (Faergeman *et al.*, 2001). Also, the acyl-CoA binding protein and the Ssn6–Tup1 complex were shown to be involved in repression of *OLE1* (Fujimori *et al.*, 1997). On the other hand, the Hap1 transcriptional activator (Choi *et al.*, 1996) and two transcription factors, Spt23 and Mga2p, which are initially synthesized as inactive ER-bound precursors, positively regulate *OLE1* expression (Zhang *et al.*, 1999). Ole1p is a naturally short-lived enzyme and is degraded by ubiquitin/proteasome-dependent ER-associated degradation (Braun *et al.*, 2002). Lowering of Ole1p activity leads to a loss of mitochondrial inheritance (Stewart & Yaffe, 1991) and disturbs the integrity of the nuclear membrane (Zhang *et al.*, 1999), although growth is not affected even if unsaturated fatty acid levels are reduced down to one-eighth of the normal level (Stukey *et al.*, 1989).

The human fungal pathogen *Candida albicans* is able to assume different growth forms, which appear to have different roles for host–cell interaction and virulence (reviewed by Ernst, 2000). At body temperatures and in the presence of inducing agents, a true hyphal form is induced, which may be involved in anchoring within and penetrating tissues, while at lower temperatures, in the absence of inducers, a unicellular yeast form is favoured (Joshi *et al.*, 1993; Sonneborn *et al.*, 1999). The yeast form, in special genetic backgrounds at low temperatures, may spontaneously form a rod-like appearance (opaque). Thick-walled chlamydospores appear in certain media, preferentially at lower temperatures. The striking dependence of temperature on the generation of morphological forms suggests a role of membrane fluidity in morphogenesis of *C. albicans*. Although some signalling pathways mediating the yeast–hypha transition have been defined, no membrane sensors are yet known which mediate environmental cues and specifically could act as cellular 'thermometers'. In this report, we characterize the *OLE1* gene of *C. albicans* and show that it is essential for viability. We show for the first time in a fungal developmental system that levels of oleic acid in cellular lipids are critical for morphogenetic competence. Thus, a modest reduction in oleic acid does not affect growth in the yeast form, but effectively prevents the formation of hyphal filaments and chlamydospores in aerobic conditions. Our results indicate, however, that overall membrane fluidity is not directly responsible for the morphogenetic potential of *C. albicans*, but that oleic acid has a specific role to activate specific morphogenetic pathways.

METHODS

Strains and growth conditions. *C. albicans* strains used in this study are listed in Table 1. Transformed strains were generated as described by Wilson *et al.* (1999). Strains were routinely grown in YPD or SD medium (Sherman *et al.*, 1986). A 1 % solution of the non-ionic detergent Igepal CA-630 (Sigma) was used for solubilization of 0·5 mM oleic acid in media (Stukey *et al.*, 1989). Strains were incubated in microaerophilic conditions by using a CampyGen bag in an anaerobic jar (Oxoid). The *PCK1* promoter (*PCK1p*) was induced in SCAA medium (0·67 % yeast nitrogen base without amino acids and 2 % Casamino acids) or SL medium (0·67 % yeast nitrogen base, 2 % sodium lactate) and repressed in SD medium (Leuker *et al.*, 1997). To repress the *MET3* promoter (*MET3p*), SD medium supplemented with different concentrations of methionine and/or cysteine was used (Care *et al.*, 1999).

Strains were grown for 3–4 days at 37 °C on Lee's medium (Lee *et al.*, 1975), or on Spider-Plates (Liu *et al.*, 1994) or on 5 % horse serum solidified by 2 % agar to induce hyphae. Corn meal agar (CMA) (Difco) containing 0·33 % Tween 80 was used for chlamydospore induction (Joshi *et al.*, 1993). Strains were streaked lightly on the agar surface and covered by coverslips. Following incubation at room temperature for 5 days, photographs of chlamydospores and filaments were taken with a Zeiss Axioscop microscope across the coverslips.

S. cerevisiae Y0779 (Δ*ole1*::*LEU2 leu2,3-112 lys2-801 trp1-1 ura3-52*), kindly provided by S. Jentsch, was cultured at 30 °C in SD medium supplemented with 0·2 % oleic acid in 0·2 % NP40 (Braun *et al.*, 2002). It was transformed by expression plasmids containing *OLE1* (pSKM24) or *OLE1-GFP* (pSKM62) under transcriptional control of the *C. albicans PCK1* promoter.

Chromosomal deletions of *OLE1* and *OLE2*. *C. albicans* sequence data were obtained from the Stanford Genome Technology Center website (http://www-sequence.stanford.edu/group/candida). The genomic region of *OLE1* was isolated by PCR using DNA of strain CAI4. The entire *OLE1* coding region was disrupted by the Ura-blaster method (Fonzi & Irwin, 1993). A cassette for disruption of *OLE1* was constructed in several steps. First, sequences 5′ and 3′ of the *OLE1* ORF were amplified by PCR and subcloned. A fragment (0·98 kb) of the 5′ sequences flanking the start of the *CaOLE1* ORF was amplified using primers Ole1disA and Ole1disB (5′-CTAGAGCTC***GGATCC*****CACGGAACTAAAC**-3′/5′-CTAGAGCTC*GGATCC***AGCAGCAATGGCATTC**-3′; bold, regions of homology; italics, *Bam*HI); similarly, 728 bp of the 3′ untranslated sequences were amplified using primers Ole1disC and Ole1disD (5′-CTAGAGCT-*CTGC***AGAAGGAAAAGCAATC**-3′/5′-CTAGAGCT*CTG***CAGGCGA-CTACATACATAC**-3′; bold, regions of homology; italics, *Pst*I). PCR fragments were subcloned into pUC18, which resulted in plasmids pSKM8 and pSKM7, respectively. The *Bam*HI fragment of pSKM8 was cloned into the *Bgl*II site of p5921. The *Pst*I fragment of pSKM7 was inserted into the *Pst*I site of the resulting plasmid pSKM55. A plasmid with *OLE1* flanking sequences in the correct orientation was obtained (pSKM56). Its *Hin*dIII fragment containing the *OLE1* disruption cassette was used to transform strain CAI4.

OLE2 was disrupted similarly by subcloning the regions flanking and partially including the ORF. The 876 bp 5′ region was amplified

Table 1. Strains and plasmids used in this study

***C. albicans* strains**	**Genotype or description**	**Reference/source**
SC5314	Prototroph	Fonzi & Irwin (1993)
CAF2-1	*URA3*/*ura3*Δ::*imm434*	Fonzi & Irwin (1993)
CAI4	*ura3*Δ::*imm434*/*ura3*Δ::*imm434*	Fonzi & Irwin (1993)
ΔO7 and ΔO8	As CAI4, but *OLE1*/*ole1*Δ::*hisG URA3 hisG*	This work
ΔO7.2 and ΔO8.2	As CAI4, but *OLE1*/*ole1*Δ::*hisG*	This work
ΔO7.2/25-2 and ΔO8.2/25-2	As CAI4, but *MET3p-OLE1*/*ole1*Δ::*hisG*	This work
cII and cIV	As CAI4, but *OLE2*/*ole2*Δ::*hisG URA3 hisG*	This work
h and 8.4	As CAI4, but *OLE2*/*ole2*Δ::*hisG*	This work
JA1 and JA2	As CAI4, but *ole2*Δ::*hisG*/*ole2*Δ::*hisG URA3 hisG*	This work
JA1x and JA2x	As CAI4, but *ole2*Δ::*hisG*/*ole2*Δ::*hisG*	This work
CA2d1m	As CAI4, but *MET3p SEC20*/*ole1*Δ::*hisG*	Weber *et al.* (2001)
***C. albicans* plasmids**		
pBI-1		Stoldt *et al.* (1997)
pSKM24	*PCK1p-OLE1* in pBI-1	This work
pSKM62	*PCK1p-OLE1-GFP* in pBI-1	This work
pSKM63	*PCK1p-myc-OLE1* in pBI-1	This work
pAP5	*PCK1p-OLE2* in pBI-1	This work

by genomic PCR using primers 709 and 710 (5′-TAT*GGATC***CAAA-ACTCCTGTAGATGG**-3′/5′-TAT*GGAT***CC**ACATACAAGACTGC**-3′; bold, regions of homology; italics, *Bam*HI); the 870 bp 3′ region was amplified by genomic PCR using primers 711 and 712 (5′-TAT*AG**ATC***TGACTGCTGCGGTG**-3′/5′-TAT*AGA**TCT***GCCAACTT-TTCTAATGC**-3′; bold, regions of homology; italics, *Bgl*II). The *Bam*HI fragment carrying the 5′ region was subcloned into the *Bgl*II site of p5921, while the *Bgl*II fragment carrying the 3′ region was subcloned into the *Bam*HI site of the resulting vector, to construct pAP9a. The *Sac*I–*Sph*I fragment of pAP9a was used for sequential disruption of both *OLE2* alleles (Fonzi & Irwin, 1993).

To place *OLE1* under control of the *MET3* promoter we followed a previously described strategy (Care *et al.*, 1999). First, a fragment of 624 bp corresponding to the 5′ end of the *OLE1* ORF was amplified by PCR using the primers Ole*BH*I-Nterm (5′-CTTAAGCTTGG**ATCC-CATGTGAGAAAACC**-3′) and Ole*StpBH*I-*Hin*dIII (5′-CTTAAGCT-TGGAT**CC**TAAGATTGCTTTTCC-3′; bold, regions of homology; underlining, *Hin*dIII, *Bam*HI) and the PCR fragment was cloned into pUC18, resulting in plasmid pSKM20. The *Bam*HI fragment of pSKM20 was inserted into the single *Bam*HI site of pCaDis, downstream of the *MET3* promoter (Care *et al.*, 1999). The resulting plasmid pSKM25 was linearized by *Nco*I (which cuts within the *OLE1* fragment) and was used to transform the heterozygous strain ΔO8.2 (*OLE1*/*ole1*Δ::*hisG*).

Overexpression of *OLE1* and *OLE2*. To overexpress *OLE1* in *C. albicans* we used pBI-1, a derivative of pRC2312 (Stoldt *et al.*, 1997) containing the *PCK1* promoter. The entire *OLE1* coding region was amplified by genomic PCR using primers OLE*Hin*dIII-BHIATG (5′-CTT*AAGCTTGGATCC*ACAATGACTACAGTTG-3′) and OLEStpBHI-*Hin*dIII (5′-CTT*AAGCTTGGATCC*TAAGATTGCTTTTCC-3′) (italics: *Hin*dIII, *Bam*HI sites) and cloned into pUC18 to generate pSKM19. The 1·072 kb *Bam*HI fragment of pSKM19 was inserted downstream of *PCK1p* into the *Bgl*II site of pBI-1, thereby generating *OLE1*-overexpression plasmid pSKM24. Similarly, the entire *OLE2* ORF was amplified by genomic PCR using primers 707 and 708 (5′-TAT*GGATCC*AGGCAAATAATATATCC-3′/5′-TAT*GGATCC*A-TTAATCTGTTAAAGTAG-3′; italics, *Bam*HI) and inserted, as a 1·58 kb fragment, into the *Bgl*II site of pBI-1 to generate the *OLE2*-overexpression vector pAP5.

For C-terminal tagging of *OLE1* with green fluorescent protein (GFP), we generated a PCR fragment containing *PCK1p-OLE1* with primers PCKp-HIII (5′-AGAAGCTT**GGCTGCAGGTCGAC**-3′) and Ole1-GFP (5′-AATAAGCTT**CCAGATTGCTTTTCCTTCTCC**-3′) (bold, regions of homology; underlined, *Hin*dIII), using pSKM24 as a template DNA and the resulting PCR fragment was cloned into pUC18 (pSKM58). The *Hin*dIII fragment of pSKM58 containing *PCK1p-OLE1* without the stop codon was cloned into the single *Hin*dIII site in-frame with *GFP* into vector pRCGFP3 to generate pSKM62. To C-terminally tag *OLE1* with a *myc* epitope, we used primers PCKp-HIII-BHI-SalI (5′-GTCGACGGATCC**AAGCTTGGCTGCAGGTCG-AC**-3′; bold, regions of homology; underlined, *Sal*I, *Bam*HI, *Hin*dIII) and Ole1-Myc (5′-AATGGATCC*TCACAAGTCTTCCT-CGGAGATTAGCTTTTGTTCACC***AGATTGCTTTTCCTTCTCC**-3′; bold, regions of homology; underlined, *Bam*HI; italics, *myc* epitope) to generate a PCR fragment, which was cloned into pUC18. The *Bam*HI fragment of the resulting plasmid pSKM59 was cloned into the *Bgl*II site of pBI-1 to generate plasmid pSKM63.

Blotting procedures. Total RNA was isolated from liquid cultures as described (Stoldt *et al.*, 1997). The conditional strain ΔO7.2/25-2 was grown in SD medium without or with 0·25 mM methionine/cysteine. Following denaturing gel electrophoresis, RNA blots were probed with the 966 bp *Hin*dIII–*Eco*RI fragment of pSKM19, which corresponds with the 5′ region of the *OLE1* ORF, or with the 938 bp *Eco*RI fragment derived from the *OLE2* ORF.

Immunoblottings for detection of Ole1p-myc fusions in crude extracts of transformants carrying pSKM63 were carried out as described (Weber *et al.*, 2001).

Fatty acid analyses. Lipids were extracted from cells disrupted by glass beads, as described (Daum *et al.*, 1999), and subjected to methanolysis using BF_3/methanol. Fatty acyl methyl esters were separated by GC on a Shimadzu GC-17A (version 3) gas chromatograph using a FS-CW-20M-0·25 polarity capillary column (0·25 mm × 25 m; film thickness 0·25 mm) with a temperature gradient (160–200 °C at 1 °C min^{-1}; hold at 200 °C for 20 min). Fatty acids were identified by comparisons to a commercial standard (RM-6; Supelco).

Determination of membrane fluidity. Cells grown to an OD_{600} value of 1 (or OD_{600} 0·7 for the conditional strain) were washed and converted to spheroplasts by zymolase 20T treatment. Spheroplasts were washed thrice in 20 mM Tris/HCl pH 7·5/10 mM $MgSO_4$/0·6 M sorbitol and resuspended in this buffer. An aqueous solution of 1,6-diphenyl-1,3,5-hexatriene (DPH) was prepared by diluting a 2 mM solution in tetrahydrofuran into 50 ml of 20 mM Tris/HCl pH 7·5 and removing traces of tetrahydrofuran by flushing with nitrogen. DPH (2 μM) was added to spheroplasts (4×10^8 cells ml^{-1}) and incubated at 30 °C in a water bath shaker for 1 h. Fluorescence polarization was determined by excitation with vertically polarized monochromatic light (360 nm) and measurements of emission intensities at 426 nm using an analyser oriented parallel or perpendicular to the excitation light (Smriti *et al.*, 1999; Kaur & Bachhawat, 1999).

The degree of fluorescence polarization (P) was calculated according to the following formula

$$P = \frac{I_{VV} - (I_{VH} \times G)}{I_{VV} + (I_{VH} \times G)}$$

where I_V is the corrected fluorescence intensity and subscripts V and H indicate the values obtained with vertical or horizontal orientation, respectively, of the polarizer and analyser (in that order). The corrected fluorescence was determined by subtracting the intensity of light measured with unlabelled control spheroplasts from the intensity observed with labelled cells. The optical components used in the instruments have particular polarizing properties causing interferences which are corrected by calculating factor *G* (called grating factor). *G* is calculated as I_{HV}/I_{HH}.

GFP fluorescence microscopy. Cells were used for fluorescence microscopy directly without fixation. Nuclei were stained by the addition of 10 μg 4′,6-diamidino-2-phenylindole (DAPI) ml^{-1} to the cell suspension. All cells were viewed using a Zeiss Axioplan 2 fluorescence microscope. Images were taken with a Quantix Digital CCD camera using METAMORPH software and processed in Corel PHOTOPAINT 11.0.

RESULTS

Identification of the *C. albicans OLE1* and *OLE2* genes

Inspection of *C. albicans* genomic sequences (http://www-sequence.stanford.edu/group/candida) revealed two genes with homology to the *S. cerevisiae OLE1* gene encoding Δ9 stearoyl-CoA desaturase, which were designated *OLE1* (orf6.6333; CA3921) and *OLE2* (orf6.5882; CA3576). The respective gene products share 33 % identity among themselves and have 57 and 32 % identity, respectively, to Ole1p of *S. cerevisiae* (Stukey *et al.*, 1989). The conceptual *C. albicans* Ole1 protein contains 486 residues, with a predicted molecular mass of 55·3 kDa, while Ole2p contains 526 residues (61 kDa). The molecular mass of Ole1p was confirmed by a *myc*-tagged derivative of Ole1p, which in immunoblottings of extracts of pSKM63 transformants revealed a single protein of about 55 kDa using an anti-*myc* antibody (data not shown). Calculation of a phylogenetic tree of fatty acid desaturases revealed close homologies among most fungal Ole1 proteins, while the *C. albicans* Ole2 protein, as well as the *Aspergillus fumigatus* Ole1 protein, were more distantly related, being situated on a common branch with mammalian desaturases (Fig. 1A).

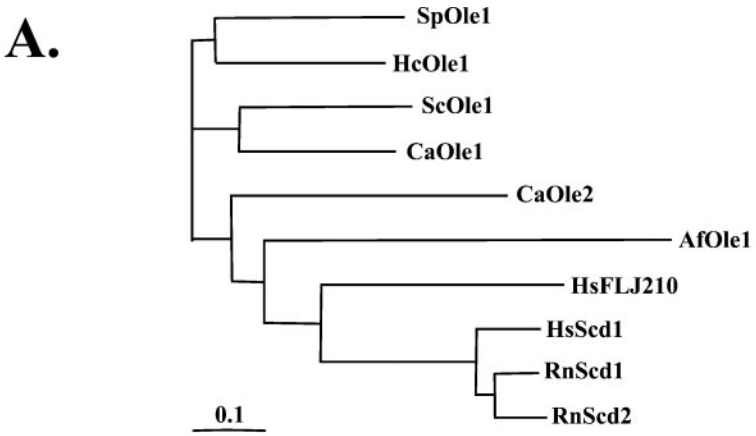

Fig. 1. Comparisons of Δ9 fatty acid desaturases. (A) A phylogenetic tree was calculated from a CLUSTAL_X alignment using the TREEVIEW software (version 1.6.5). Bar, 0·1 amino acid substitutions per site. Ole1-type proteins of *Saccharomyces cerevisiae* (Sc), *Schizosaccharomyces pombe* (Sp), *Histoplasma capsulatum* (Hc), *Aspergillus fumigatus* (Af), *Homo sapiens* (Hs) and *Rattus norvegicus* (Rn) were compared. (B) Homology among fatty acid desaturase signature regions. The fatty acid desaturase signature V sequence is underlined. Identical residues are shaded grey and highly conserved residues are marked with an asterisk.

C. albicans Ole1p contains blocks of homology compared to desaturase family 1 members, such as signature I sequences between residues 93–110 and 139–159, as well as signature V sequences between residues 309 and 323 (Fig. 1B). As with other fungal, but not mammalian, desaturases, Ole1p and Ole2p contain integral cytochrome b_5 domains between residues 407–440 and residues 438–467, respectively. Computer analysis using the TMPRED program (http://www.ch.embnet.org/software/TMPRED_form.html) predicts at least four transmembrane regions able to form two pairs of transmembrane regions, consistent with the current model for the topology of ScOle1p (Stukey *et al.*, 1990).

Disruption of *OLE1* and *OLE2* alleles

Derivatives of the wild-type strain CAI4 were constructed which contained different disrupted *OLE1* alleles (Fig. 2A). Heterozygous *OLE1*/*ole1* strains such as strain ΔO8 were generated without difficulty, while the construction of a homozygous *ole1*/*ole1* strain failed repeatedly. This result suggested that *OLE1* in *C. albicans* is essential, as is *ScOLE1* in *S. cerevisiae* (Stukey *et al.*, 1989). To confirm this hypothesis we modified the remaining intact copy of *OLE1* in the heterozygous strain ΔO8-2 by placing its ORF under transcriptional control of the *MET3* promoter, which is repressed by methionine and/or cysteine (resulting

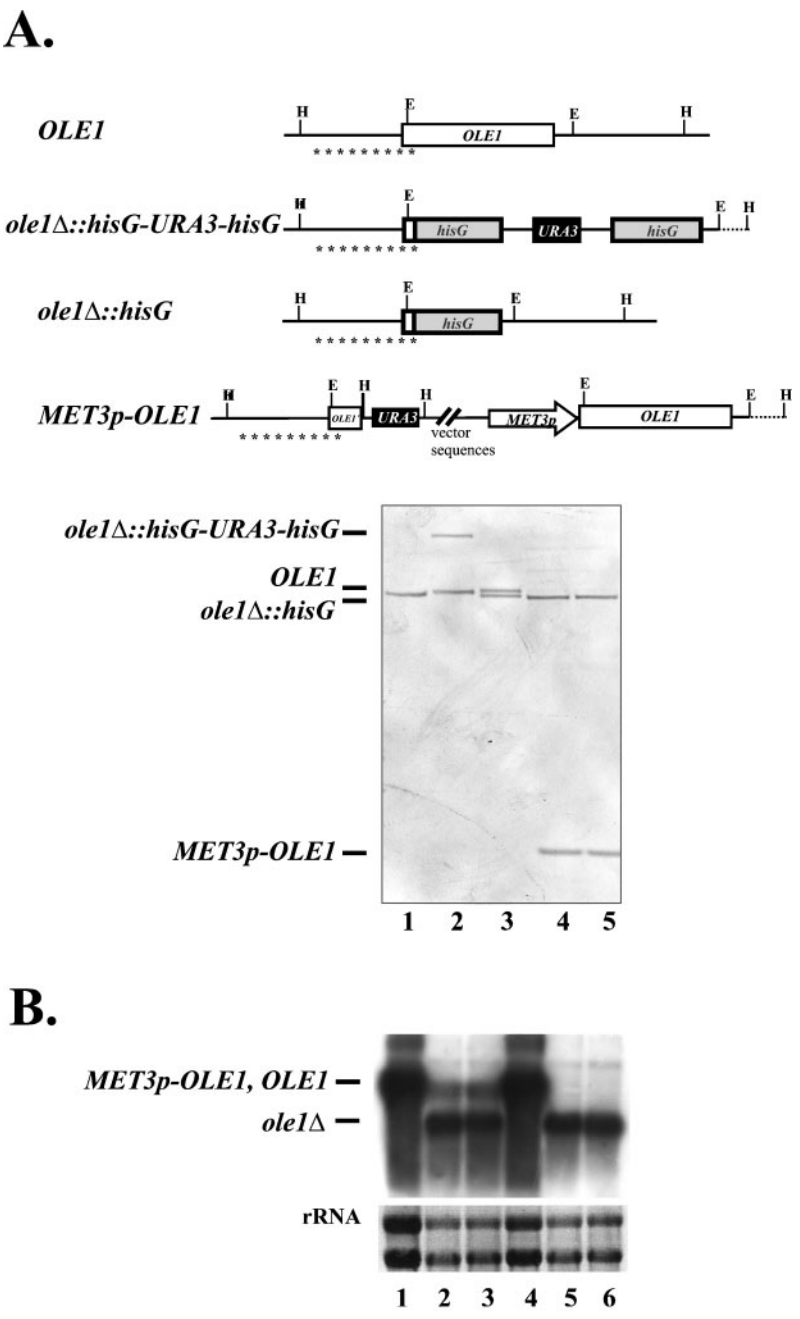

Fig. 2. *OLE1* alleles and transcripts. (A) Top, genomic configuration of the *C. albicans OLE1* wild-type locus and its deleted derivatives. H, *Hin*dIII; E, *Eco*RV; asterisks indicate the probe used in the corresponding Southern blot. Genomic DNA of strains was cut by *Hin*dIII and analysed by Southern blotting (bottom). Strains tested were: lane 1, CAI4 (*OLE1*/*OLE1*); lane 2, ΔO8 (*OLE1*/*ole1*Δ::*hisG-URA3-hisG*); lane 3, ΔO8-2 (*OLE1*/*ole1*Δ::*hisG*); and lanes 4 and 5, ΔO8.2/25-2 (*MET3p*::*OLE1*/*ole1*Δ::*hisG*). (B) *OLE1* transcripts. Total RNA of strain CAF2-1 (lanes 1 and 4) and of the conditional strain ΔO7.2/25-2 (lanes 2, 3 and 5, 6) was analysed by Northern blotting, using a probe homologous to the *OLE1* ORF. Strains were grown in the absence (lanes 1–3) and in the presence (lanes 4–6) of 0·25 mM methionine/cysteine. The positions of the *MET3p-OLE1* and *OLE1* transcripts, as well as of the truncated Δ*ole1* transcript, are indicated. rRNA stained by ethidium bromide was used as loading control.

strains ΔO7.2/25-2 and ΔO8.2/25-2). The genomic configuration of two independently constructed lineages of homozygous, heterozygous and conditional strains was verified by Southern blottings as exemplified in Fig. 2(A). Pairs of isogenic mutant strains were identical in all phenotypes, as described below.

To confirm that *OLE1* in strain ΔO7.2/25-2 was under transcriptional control of the *MET3* promoter, we performed a Northern analysis of total RNA of this strain, grown without or with limiting amounts (0·25 mM) of methionine (see below). In the absence of methionine an *OLE1* transcript was detected (Fig. 2B, lanes 2, 3), which was missing in the presence of methionine (lanes 5, 6). The size of this transcript of 2 kb corresponded to the *OLE1* transcript in a control strain (lanes 1, 4). Growth in the presence of methionine did not influence the authentic *OLE1* transcript and, in agreement, also did not regulate the expected shortened *ole1*Δ transcript of about 1·8 kb in the conditional strain, which arose by chromosomal integration of the *MET3p-OLE1* plasmid (Care *et al.*, 1999).

Using the above-mentioned procedure we also generated two independent lines of CAI4 derivatives, in which one or both *OLE2* alleles were disrupted (data not shown). At the *OLE2* locus strains cII and CIV are heterozygous (*ole2*/*OLE2*), while strains JA1 and JA2 are homozygous mutants (*ole2*/*ole2*). *URA3*-minus derivatives of strain JA1 were isolated by FOA selection (resulting strains JA1x and JA2x), which subsequently were transformed by the expression vector pAP5 to reconstitute *OLE2*. In Northern blottings using an *OLE2* probe on total RNA of strain CAF2-1, we observed that *OLE2* was expressed, generating a transcript of about 2 kb, but signal intensity was much lower compared to the *OLE1* transcript (data not shown). Thus, *OLE2* appears to be expressed at low levels, at least in the conditions used here.

Growth depends on *OLE1* expression levels

The conditional strain ΔO8.2/25-2 (*MET3p-OLE1*/*ole1*Δ::*hisG*) was streaked on SD medium containing cysteine and/or methionine to repress *OLE1* (Fig. 3A). Growth was blocked completely at 2·5 mM cysteine, 0·25 mM methionine and 0·05 mM of a cysteine/methionine mixture, while the heterozygous strains ΔO7 and ΔO8 were not affected. Likewise, in liquid SD medium containing 2·5 mM methionine/cysteine, growth of the conditional strains was blocked completely, with a terminal phenotype of mostly unbudded cells that tended to aggregate (data not shown), suggesting a block in the G_1 or G_0 phase of the cell cycle. The threshold level, at which methionine/cysteine blocked growth, strongly depended on the type of media and growth conditions used: whereas cysteine/methionine at a concentration of 0·05 mM prevented growth on solid SD medium, it did not block growth in liquid SD medium up to 0·25 mM cysteine/methionine. Also, on Lee's medium, growth of the conditional strain was inhibited significantly only at methionine concentrations above 5 mM. This medium dependence may be due to different efficiencies of *MET3p* repression in different conditions. The addition of 0·5 mM oleic acid in 1 % Igepal CA-630 as solubilizer was able to restore growth of the conditional strain ΔO8.2/25-2 in *OLE1*-repressing conditions (Fig. 3B). In contrast, the conditional control strain CA2d1m, which contains the essential *SEC20* gene under control of *MET3p* (Weber *et al.*, 2001), could not be rescued by oleic acid. Thus, these results indicate that *OLE1* is essential in *C. albicans* and suggest that it is involved in oleic acid biosynthesis.

To further confirm the role of Ole1p as a fatty acid desaturase, we attempted to complement the *S. cerevisiae ole1* mutant Y0779, which lacks this activity (Braun *et al.*, 2002), by the *C. albicans OLE1* gene. Expression vector pSKM24 contains *CaOLE1* under transcriptional control of the *C. albicans PCK1* promoter, the *CARS1* replicator and the *CaURA3* gene, which we found to be functional in the heterologous host *S. cerevisiae*. Transformants of strain Y0779 containing pSKM24 were able to grow on SD medium in the absence of added oleic acid, while transformants with the control vector pBI-1 did not grow (Fig. 3C). A similar result was obtained using medium containing 2 % galactose as the carbon source, indicating that the heterologous *C. albicans PCK1* promoter is active in *S. cerevisiae* in the presence of glucose. Taken together, these results strongly suggest that the *C. albicans OLE1* gene encodes Δ9 stearoyl desaturase activity.

In contrast to the results obtained for *OLE1*, both alleles of *OLE2* could be deleted without any difficulty and the resulting mutants (e.g. strain JA2) grew as well as the wild-type in all media, in the absence of oleic acid. Furthermore, the *CaOLE2* expression vector pAP5 was unable to reconstitute the *S. cerevisiae ole1* mutant Y0779. Thus, these experiments provided no evidence for a function of the *OLE2* gene product as a Δ9 stearoyl desaturase.

Hyphal morphogenesis depends on *OLE1* expression levels

We examined, next, if partial repression of *OLE1* expression in the conditional strain would still allow growth, but prevent hyphal morphogenesis. Common media used for induction of hypha formation already contain methionine and/or cysteine: Lee's medium contains 0·075 mM methionine (Lee *et al.*, 1975), which in SD medium blocks growth of the conditional strain ΔO8.2/25-2 completely (Fig. 3A); Spider and serum media contain complex sources of nitrogen likely to include methionine or cysteine. As stated above, growth of the conditional strain was not affected on Spider medium or on Lee's medium compared to the wild-type strain. In contrast, hypha formation on both media was completely blocked (Fig. 4) and microscopy revealed that colonies consisted entirely of yeast-form cells. The importance of a sufficient dosage of wild-type *OLE1* expression was confirmed by the

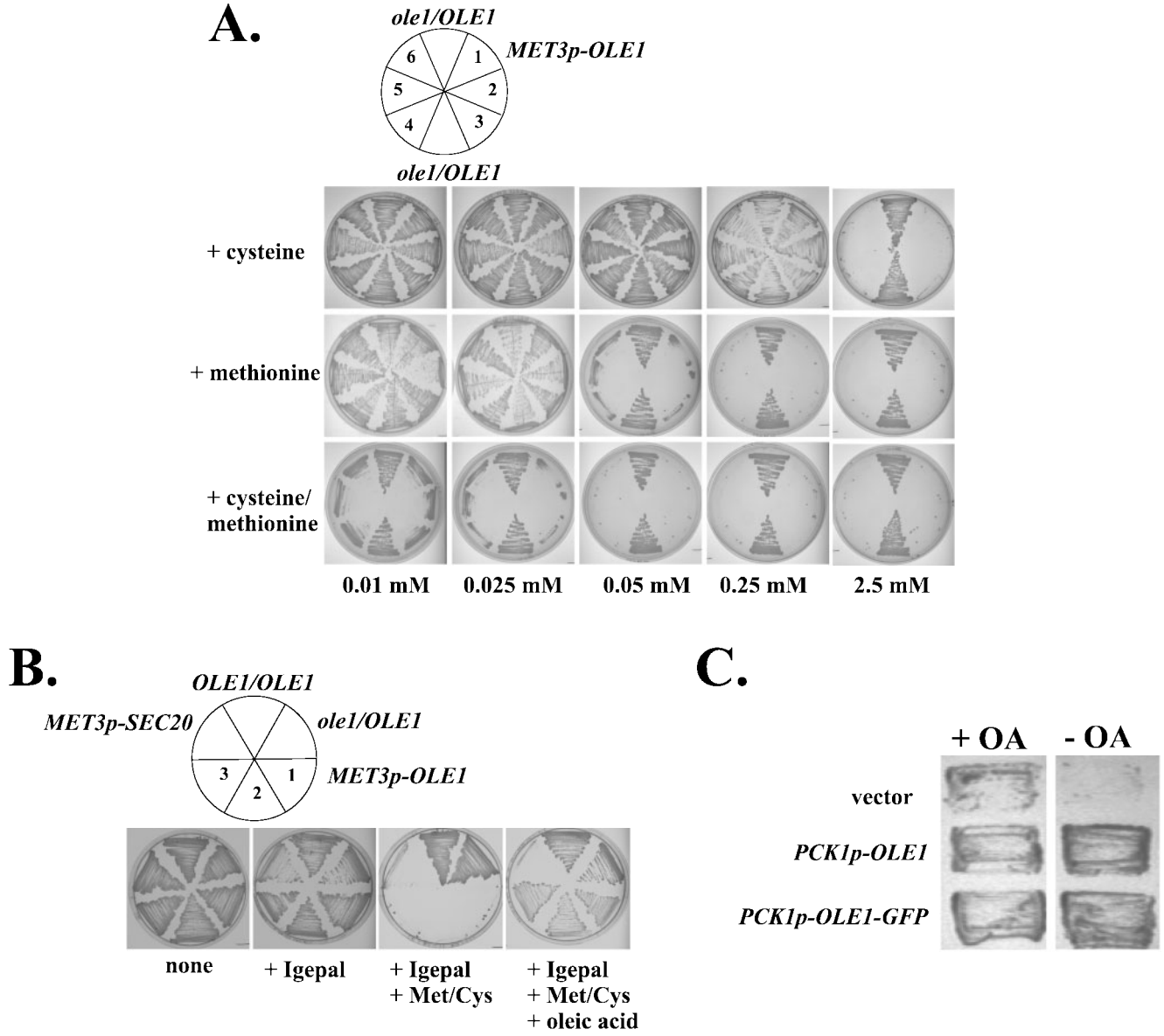

Fig. 3. Growth of the *C. albicans ole1* conditional mutants. (A) Six independent isolates of conditional *ole1* mutants (*MET3p*::*OLE1*/*ole1*Δ::*hisG*) were tested for growth at 30 °C on SD medium containing the indicated concentrations of cysteine and methionine (sectors 1–6); the heterozygous strains ΔO7 and ΔO8 (*OLE1*/*ole1*Δ::*hisG-URA3-hisG*) were used as controls. (B) Oleic acid complementation of the conditional *ole1* mutant. Isolates of mutant ΔO8.2/25-2 (sectors 1–3) were grown on SD medium containing 1 % Igepal, 2·5 mM methionine/cysteine (Met/Cys) and 0·5 mM oleic acid as indicated. As controls the wild-type CAF2-1 (*OLE1*/*OLE1*), the heterozygous strain ΔO8 (*OLE1*/*ole1*Δ::*hisG-URA3-hisG*) and the conditional mutant CA2d1m (*MET3p-SEC20*/*ole1*Δ::*hisG*) were tested. (C) Complementation of the *ole1* mutation of *S. cerevisiae*. Transformants of the *S. cerevisiae* strain Y779 (*ole1*) carrying an empty vector (pBI-1), a vector expressing *PCK1p-OLE1* (pSKM24) or *PCK1p-OLE1-GFP* (pSKM62) were streaked out on SD medium lacking uracil with or without 0·2 % oleic acid (OA) and incubated for 3 days at 30 °C.

OLE1/*ole1* heterozygous strain, which had a reduced ability to form hyphae. A more complex phenotype was observed on serum medium, on which the conditional and the heterozygous strains produced hyphae, although their growth was retarded (presumably because of methionine/cysteine as well as traces of oleic acid in serum). The effect of lowered *OLE1* expression on hyphal morphogenesis was confirmed in liquid SD medium containing 5 % serum and methionine/cysteine, although these experiments were hampered by the fact that both amino acids impaired hypha formation partially even in the control strain CAF2-1 (data not shown).

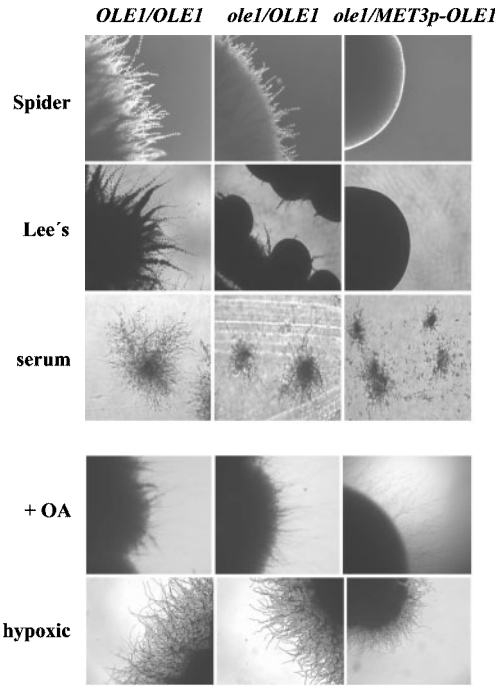

Fig. 4. Filamentation defect of *ole1* conditional mutants. Strains were grown on Spider or Lee's medium or on agar containing 5% horse serum. Strains CAF2-1 (*OLE1*/*OLE1*), the heterozygous strain ΔO8 (*OLE1*/*ole1*Δ::*hisG-URA3-hisG*) and the conditional mutant ΔO8.2/25-2 (*MET3p*::*OLE1*/*ole1*Δ::*hisG*) were tested. Following 5 days growth at 37 °C, colony phenotypes were recorded microscopically (magnification 2·5-fold). Restoration of the filamentation defect was obtained on Lee's medium in the presence of 0·5 mM oleic acid (OA) or in hypoxic conditions generated in an anaerobic jar.

The addition of oleic acid to Spider medium restored hyphal morphogenesis of the conditional strain (Fig. 4), suggesting that the lack of oleic acid was the reason for the defective morphogenetic phenotype. Surprisingly, hyphal morphogenesis was also partially restored in microaerophilic (hypoxic) conditions, in which an alternative signalling pathway leading to hyphal morphogenesis is activated (Brown *et al.*, 1999; Sonneborn *et al.*, 1999). This result suggested that lowered oleic acid biosynthesis does not change the ability to form hypha *per se*, but rather affects the induction of hypha formation.

Since these experiments had shown that low oleic acid levels could prevent morphogenesis, we also asked if increased levels would enhance hypha formation. Therefore, we pregrew strain CAI4(pSKM24) in inducing SCAA medium to overexpress *OLE1* by the *PCK1* promoter. Although in this medium a small fraction of the transformant cells (about 5%) formed elongated abnormal filaments resembling pseudohyphae, no typical true hyphae were detected. When 10% horse serum was added and cells were incubated at 37 °C, they began to form true hyphae with similar kinetics as transformant cells which had been grown in *PCK1p*-repressing S4D medium and as control cells carrying empty vector pBI-1 (data not shown). Germ tubes were of identical lengths in all strains, suggesting that different Ole1p enzyme levels were not correlated with induction and elongation of hyphae. To confirm that the growth conditions had indeed led to an overproduction of Ole1p, we grew transformant CAI4(pSKM63), producing a *myc*-tagged version of Ole1p, in identical conditions, and by immunoblottings could verify that Ole1p production was increased in SCAA-grown cells five- to tenfold, as compared to S4D-grown cells (data not shown).

Thus, these results indicated that a minimal dosage of Ole1p and of oleic acid is crucial to allow efficient hypha formation in several but not all conditions known to induce hyphae in *C. albicans*. In contrast to these results, an *ole2* deletion strain (JA2) was able to form true hyphae as was the wild-type in all conditions tested, suggesting that Ole2p does not have an essential role in hyphal morphogenesis.

Chlamydospore formation requires wild-type levels of *OLE1* expression

Because of the requirement for *OLE1* in hypha formation, we also considered the possibility that another morphogenetic event in *C. albicans*, chlamydospore formation, would be affected by *OLE1* expression. Therefore, we streaked the conditional *MET3p-OLE1* strain onto CMA containing a low level of methionine/cysteine and covered cells by a coverslip to generate microaerophilic conditions (Joshi *et al.*, 1993; Sonneborn *et al.*, 1999). Following 5 days incubation at 25 °C, chlamydospores of all strains were visible in medium lacking methionine/cysteine, while only the wild-type and the heterozygous strain produced chlamydospores in the presence of methionine/cysteine (Fig. 5). The conditional strain grew equally as well in this condition as the wild-type and heterozygous strains, but failed to form chlamydospores and this defect remained even after prolonged incubations. In addition to this defect, the conditional strain formed pseudohyphae that aggregated strongly, which was a phenotype not seen in the heterozygous and wild-type strains. Thus, wild-type expression levels of *OLE1* and corresponding levels of oleic acid are necessary to allow chlamydospore formation in *C. albicans*. In contrast to these results, strains JA1 and JA2 were able to form chlamydospores at normal levels, indicating that Ole2p is not involved in chlamydospore formation.

Intracellular localization of Ole1p

To examine the intracellular location of Ole1p, we examined a transformant carrying pSKM62, which expresses a fusion of *OLE1* to the *GFP* gene under transcriptional control of the *PCK1* promoter. Complementation of a *S. cerevisiae ole1* mutant by pSKM62-encoded Ole1-GFP demonstrated that this fusion is a functional stearoyl CoA desaturase (Fig. 3C). In transformants grown in *PCK1p*-inducing medium, green fluorescence was observed throughout the

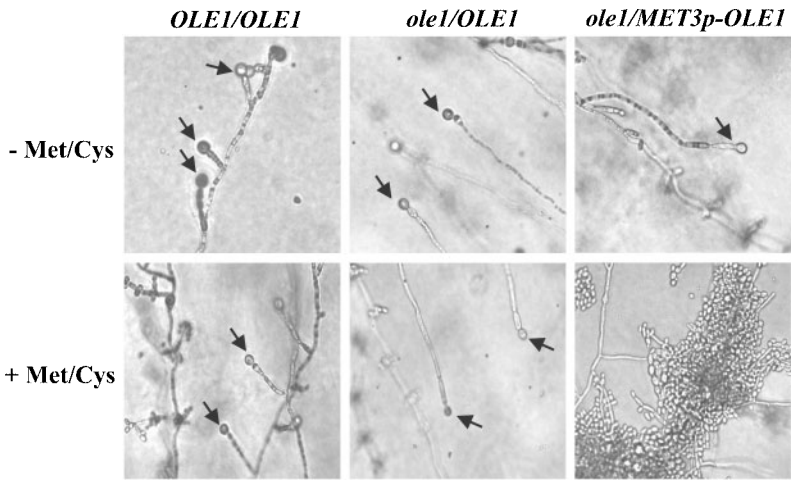

Fig. 5. Defective chlamydospore formation of the conditional *ole1* mutant. Strains were streaked out lightly on chlamydospore induction medium (CMA) without or with 0·25 mM methionine/cysteine, covered by a coverslip and incubated for 4–5 days at 25 °C. Strains used were CAF2-1 (*OLE1/OLE1*), ΔO8 (*OLE1/ole1Δ::hisG-URA3-hisG*) and ΔO8.2/25-2 (*MET3p::OLE1/ole1Δ::hisG*). Photographs of chlamydospores and filaments were magnified 100-fold (across coverslips on plates).

cell, but it was especially seen at a location surrounding the nucleus marked by DAPI staining (Fig. 6). This staining pattern is consistent with the localization of Ole1p in the ER membrane and agrees with the localization of the homologous protein in *S. cerevisiae* (Stukey *et al.*, 1990). However, because at low expression levels (i.e. in S4D medium) the transformants did not show any fluorescence, we cannot exclude the possibility that high levels of Ole1-GFP biosynthesis had an influence on its intracellular distribution.

Fatty acid analyses

To prove the effects of altered *OLE1* or *OLE2* expression, we determined fatty acid compositions of the conditional *MET3p-OLE2* strain, the *ole2* mutant and of transformants carrying overexpression vectors. In pre-tests we observed that fatty acid compositions were strongly dependent on growth media used, in agreement with a previous study on *S. cerevisiae* (Chatterjee *et al.*, 2001). For example, growth in SD medium led to a strong increase in $C_{16:0}$ and a strong decrease in $C_{18:2}$ and $C_{18:3}$ fatty acids compared to growth in SCAA or SD/methionine/cysteine medium. Therefore, to exclude medium effects, we compared mutant or overexpression strains following growth in the *same* medium.

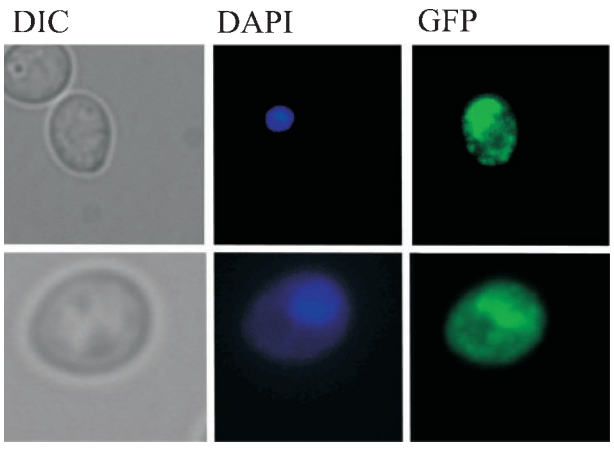

Fig. 6. Intracellular localization of an Ole1p-GFP fusion. A transformant of strain CAI4 carrying pSKM62 (*PCK1p-OLE1-GFP*) was grown in *PCK1p*-inducing SCAA medium. Cells were stained with DAPI and analysed by fluorescence microscopy in a Zeiss Axioplan 2 microscope. The differential interference contrast (DIC) image is shown along with the DAPI and GFP labellings.

The conditional mutant ΔO7.2/25-2 and the control strain CAF2-1 were pre-grown in SD medium and then inoculated into SD medium containing low amounts of methionine and cysteine (0·25 mM). At these levels, the conditional strain downregulated *OLE1* to an extent to prevent hypha formation, but to still allow growth. Following about three cell doublings, we isolated lipids and generated fatty acid methyl esters, which were analysed by GC. Representative results indicate that levels of $C_{18:1}$ (oleic) acids were reduced in the conditional strain, while the $C_{18:0}$ precursor was strongly increased relative to amounts in the control strain (Table 2). On the other hand, levels of $C_{18:2}$ and $C_{18:3}$ acids were identical in the control and conditional strains, suggesting that lowering of *OLE1* expression primarily affects the $C_{18:0}$ to $C_{18:1}$ conversion. We also note that the levels of $C_{16:0}$ and $C_{16:1}$ acids were diminished in the conditional strain. In contrast, fatty acid composition in the *ole2* deletion strain JA2 appeared similar to the control strain CAF2-1 (Table 2).

We also checked if strains carrying overexpression vectors for *OLE1* or *OLE2* would show altered fatty acid compositions compared to a control strain transformed with an empty vector. A transformant with the *OLE1*-overexpression vector pSKM24 showed only a slight increase in $C_{18:1}$ levels compared to the control, while contents of $C_{18:2}$ and $C_{18:3}$ fatty acids were not altered (Table 2). A similar pattern including an increase in $C_{18:1}$ was obtained in a strain carrying the *OLE2*-overexpression vector pAP5. The latter result is the only evidence that

Table 2. Fatty acid composition and membrane fluidity

Strain	Growth medium	Percentage of fatty acids								Membrane fluidity (P-value)
		$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Other	
CAF2-1	SD + M/C*	0	28·2	7·3	5·3	17·2	28·6	11·4	2	0·131 ± 0·008
ΔO7.2/25-2 (*MET3p-OLE1*)	SD + M/C*	1·4	19·8	2·4	20·1	11·6	31·7	12·8	0·2	0·189 ± 0·016
CAF2-1	SCAA	0	18·1	9·8	6·5	23·2	32·3	10·1	0	0·145 ± 0·009
JA1 (Δ*ole2*)	SCAA	0	15·7	14	2·5	24·8	29	9·4	4·5	0·170 ± 0·013
CAI4(pBI-1)	SCAA	0·7	19·5	10·6	4·6	23·9	31·7	9·0	0	0·184 ± 0·002
CAI4(pSKM24) (*PCK1p-OLE1*)	SCAA	0·7	17·6	11·4	3·8	27·9	28·8	9·1	0·7	0·257 ± 0·003
CAI4(pAP5) (*PCK1p-OLE2*)	SCAA	0·8	18·7	11·7	3·5	28·8	27·5	8·5	0·5	0·185 ± 0·009

*SD medium containing methionine and cysteine (0·25 mM).

OLE2, at least at elevated expression levels, may function as a Δ9 stearoyl desaturase.

Membrane fluidity measurements

It appeared possible that lowering of membrane oleic acid would lead to decreased membrane fluidity. Alternatively, we speculated that cells would cope with alterations in oleic acid levels by compensatory alterations in membrane lipids and/or proteins, which would maintain membrane fluidity at a relatively constant level. To decide between these alternatives, we measured membrane fluidity in strains with altered *OLE1* expression levels, by detection of the mobility of the fluorochrome 1,6-diphenyl-1,3,5-hexatriene, using fluorescence polarization measurements. Results were expressed as P-values, which at low values indicate high fluorochrome mobility, i.e. high fluidity, whereas elevated values indicate decreased membrane fluidity.

Partial repression of *MET3p-OLE1* in the conditional strain ΔO7.2/25-2 led to increased P-values, indicating a decrease in fluidity, as expected for a decrease in oleic acid (Table 2). On the other hand, deletion of *OLE2* also decreased fluidity, although levels of unsaturated fatty acids were very similar in the *ole2* mutant and the control strain. Furthermore, membrane fluidity was unaltered in a strain with an *OLE2*-overexpression vector and fluidity was lowered rather than increased in a transformant carrying an *OLE1*-overexpression plasmid relative to the control. Thus, we did not detect any correlation between fatty acid composition and membrane fluidity. A complicating factor in these studies was the fact that membrane fluidity depended on the type of growth medium, because CAF2-1 control cells grown in SCAA and SD medium had different fluidities (P-values of 0·145 and 0·131, respectively); furthermore, membrane fluidity in the control CAI4-transformant was unusually low (P = 0·184).

Since strains with different levels of overall membrane fluidity were obtained, we tested a possible correlation between membrane fluidity and the ability to form hyphae. In spite of the significantly enhanced membrane rigidity of the *OLE1*-overexpression strain, it was able, upon addition of 10 % serum, to form hyphae with about equal kinetics compared to control cells. The different rigidities of the control strains grown in SCAA medium or SD medium also did not alter the ability of cells to form hyphae. Furthermore, the addition of the fluidizer benzyl alcohol (Horvath *et al.*, 1998) to wild-type cells did not change the response to hypha-inducing agents (data not shown). Thus, overall membrane fluidity *per se* does not appear to be a crucial factor to establish morphogenetic competence, whereas levels of individual membrane components such as oleic acid, which may occur in membrane subdomains or 'rafts', may be relevant.

DISCUSSION

In this study, we characterized a first gene determining fatty acid metabolism in the human fungal pathogen *C. albicans*. The conclusion that *OLE1* encodes a stearoyl desaturase required for the biosynthesis of oleic acid ($C_{18:1}$) is based on (a) its high homology to such desaturases in other organisms and especially in *S. cerevisiae*, (b) its ability to complement an *ole1* mutation in *S. cerevisiae*, (c) rescue of growth and morphogenetic phenotypes occurring during low *OLE1* expression by external oleic acid, and (d) higher levels of oleic acid in an *OLE1* overexpression strain and lowered levels in a conditional strain, in which *OLE1* was downregulated. In the latter experiment, levels of $C_{18:2}$ and $C_{18:3}$ fatty acids, which do not occur in *S. cerevisiae*, were not downregulated, suggesting that $C_{18:1}$ produced by *OLE1* is not the direct precursor of $C_{18:2}$ or $C_{18:3}$ acids, but that a separate desaturase is involved. We suspected that the *OLE2* gene described here would fulfil this function. The *OLE2* gene product shares relatively low homology with *OLE1* of yeast-like fungi, but has greater homology to genes encoding desaturases of filamentous fungi and mammals. However, in an *ole2* deletion strain the pattern of fatty acids was similar to a wild-type strain, ruling out a function of Ole2p in the generation of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ fatty acids. Interestingly, a strain overexpressing *OLE2* showed elevated oleic acid levels, which we take as a hint of a possible desaturase function of Ole2p, which is

detectable at high production levels. It is possible that *OLE2* is involved in the synthesis of other fatty acid derivatives, such as leukotrienes and prostaglandins, in *C. albicans* (Noverr *et al.*, 2002). The task of generation of $C_{18:2}$ and $C_{18:3}$ fatty acids may be assumed by the gene products of two ORFs (orf6.1443; orf6.5913) in the *C. albicans* genome, which encode proteins that are highly homologous to Δ12 fatty acid desaturases of *Aspergillus nidulans* (Calvo *et al.*, 2001).

Complete repression of *OLE1* prevented growth, indicating that Ole1p and its product oleic acid provide essential functions. We presume that oleic acid is also needed for specific functions, such as for oleic acid-dependent membrane sensors, and that it has a role in regulating overall and localized membrane fluidity. Low levels of *OLE1* expression did not impair growth of the yeast form, but significantly blocked hyphal morphogenesis on solid and in liquid induction media. A specific set of signalling pathways leading to hyphal morphogenesis is known to be required in most induction conditions in the presence of oxygen (Ernst, 2000), while in embedded hypoxic conditions an alternative signalling pathway, which is down-regulated in wild-type cells, is operative (Brown *et al.*, 1999; Sonneborn *et al.*, 1999). Because low *OLE1* expression permitted hypha formation in embedded/hypoxic conditions but prevented filamentation in aerobic conditions, it appears likely that threshold levels of oleic acid are required specifically for the function of the aerobic pathways. Thus, oleic acid does not appear to have a general role in filament formation, for example for late events in hyphal development, but it is implicated in early events of activation of specific signalling pathways. Conceivably, because the biosynthesis of oleic acid requires oxygen, it could be a signalling molecule activating (yet unknown) membrane sensors transmitting external cues to internal pathways operative in aerobic conditions.

It is known that elevated temperatures increase membrane fluidity, while lowered temperatures decrease fluidity. Because elevated temperatures are a decisive environmental cue to trigger hyphal morphogenesis in *C. albicans*, we speculated that the state of its membrane fluidity could act as a cellular 'thermometer' signalling directly to morphogenetic pathways. It has indeed been reported that stress responses and in particular the heat-shock responses in yeasts are activated by membrane perturbations (Carratu *et al.*, 1996; Moskvina *et al.*, 1999). Lowering *OLE1* expression in *C. albicans* indeed led to decreased membrane fluidity, as expected for a direct role of membrane fluidity in adjusting the set point of temperature induction of hyphal growth. However, further findings provide arguments against a direct correlation between membrane fluidity and morphogenesis: (1) elevated temperatures did not restore hypha formation in the *MET3p-OLE1* conditional strain, (2) no correlation between membrane fluidity and hyphal induction was detected, (3) benzyl alcohol, a membrane fluidizer, did not increase hypha formation, and (4) overexpression of *OLE1* led to a slightly increased level of oleic acid, but strongly increased membrane rigidity, which nevertheless did not interfere with hypha formation. Increased rigidity in the latter experiments may be due to compensatory increases in other membrane components, for example an increase in ergosterol levels, which may maintain the physical state of membranes constant. According to a similar principle, it has been described that the composition of the *C. albicans* cell wall is subject to compensatory alterations (Kapteyn *et al.*, 2000). Furthermore, a genome-wide transcriptional profiling of genes induced during hyphae induction recently revealed that a heat shock alone is not able to induce hyphae-specific genes and hyphal morphogenesis (Nantel *et al.*, 2002). Thus, we favour a model in which stress responses and induction of morphogenesis do not share the same dependence on membrane fluidity. We rather postulate that levels of oleic acid have a direct effect on specific components of the hyphal induction machinery.

In addition to the defect in hyphal morphogenesis, the development of chlamydospores was blocked at low *OLE1* expression levels. Although the functions of the thick-walled chlamydospores in the biology and virulence of *C. albicans* are currently unclear, it is evident that their development requires a specific morphogenetic pathway, which depends on oleic acid. The finding of a threshold level of oleic acid for morphogenetic events in *C. albicans* suggests that the reason for defective phenotypes in several morphogenetic mutants of *C. albicans* may be an impaired lipid and/or fatty acid and/or oleic acid metabolism. In agreement with this notion, in *S. cerevisiae* the Tup1 regulator represses transcription of *OLE1* (Fujimori *et al.*, 1997) and *C. albicans tup1* mutants grow in a pseudohyphal form (Braun & Johnson, 1997), while we observed that *OLE1* overexpression favours an abnormal pseudohyphal growth form, although only in a small fraction of cells. Our results further suggest that Ole1p may be a suitable target for future antifungal agents. Because *OLE1* is essential, it is likely that potential Ole1 inhibitors will prevent cell growth and lead to a rapid loss of viability. This would be an advantage compared to azole inhibitors of ergosterol biosynthesis, which do not kill fungal pathogens. Even at low doses of inhibitors, which reduce but do not eliminate Ole1 function, hyphal morphogenesis and consequently virulence of *C. albicans* would be blocked. A major structural difference between mammalian and fungal Ole1 proteins is the presence of an integral cytochrome b_5 domain in fungal desaturases, which may allow the development of selective inhibitors.

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