Changes in cell morphology and carnitine acetyltransferase activity in *Candida albicans* following growth on lipids and serum and after *in vivo* incubation in mice

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***Candida albicans* C316, maintained in the yeast form, showed a proliferation of peroxisomes when grown on triolein or serum as sole carbon source but these structures were absent from glucose-grown cells. Peroxisomes were also apparent in *C. albicans* obtained after injection into mice and recovery from intraperitoneal washings and kidneys; they may therefore be useful markers to assess a potential *in vivo* response in cells that are growing *in vitro*. Trans-cell-wall structures also occurred in *C. albicans* grown on triolein or serum, and in cells cultured *in vivo*, but were not seen in cells grown on glucose. These structures consisted of electron-dense fibrillar material penetrating through the cell wall from the plasmalemma side and protruded out to the exterior of the cell. Endoplasmic reticulum, located at the periphery of the cell, was found to be in close proximity with these cell wall structures. Carnitine acetyltransferase (CAT; EC 2.3.1.7), the key enzyme for the translocation of acetyl units between intracellular compartments, was present in low activities in glucose-grown cells; its activity was increased some 100-fold in triolein-grown cells but only 4-fold in serum-grown cells. It was not possible to assess this activity in the *in vivo*-cultured cells. Two separate CAT proteins, partially purified from isolated microchondria and peroxisomes, respectively, were identified, with different specificities and kinetic properties.**

Keywords: *Candida albicans*, carnitine acetyltransferase, lipid, mitochondrion, peroxisome

INTRODUCTION

The phenotypic switch between yeast and hyphal form, thought to be involved in increased adherence and host tissue invasion *in vivo* (see Odds, 1988), has been observed in pathogenic *Candida* species grown on substrates as apparently diverse as hydrocarbons and serum. Fukui's group found that *C. tropicalis* grown on n-alkanes often grew in the filamentous form (Yamamura *et al.*, 1975; Hirai *et al.*, 1972) and Hitchcock *et al.* (1989), Gow & Gooday (1984) and Shepherd *et al.* (1980) have found that serum is consistently effective in the induction of germ-tube formation and hyphal growth with *C. albicans*.

The relationship between pH, temperature and the morphology of *C. albicans* has also been exploited to study yeast and filamentous forms without changing major components of the medium (Prasad, 1987). Yet, even if the morphology is maintained, the nutritional status of the yeast must elicit changes in its ultrastructure. Compartmentalization and subcellular localization of enzymes appear to play a crucial role in control of metabolite pool sizes, substrate cycling and flux through metabolic pathways (Crabtree *et al.*, 1990; Sumegi *et al.*, 1990; Srere *et al.*, 1990) and can vary according to the growth substrate being used by the yeast (for examples see Veenhuis & Harder, 1987, 1991). Similarly, as we record here, a complex physical and nutritional environment *in vivo* can also elicit extreme intracellular and morphological changes in *C. albicans* C316, and consequently, we have investigated the contribution that complex carbon sources, such as lipid and serum proteins, may have in inducing similar morphological changes *in vitro*. For

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Abbreviations: CAT, carnitine acetyltransferase; IP, intraperitoneal; TCA, tricarboxylic acid.

example, the appearance of peroxisomes is engendered by growing the cells on triolein and serum. Triolein is degraded by the organism via the β -oxidation cycle, the enzymes of which are located wholly within the peroxisomes (Tanaka & Fukui, 1989; Veenhuis & Harder, 1991) and, similarly, many of the amino acids derived from serum are also handled in the peroxisomes. A common feature of degradative pathways in the peroxisomes is their linkage to catalase/peroxidase and also to the formation of acetyl-CoA. In *C. albicans*, the subsequent movement of acetyl-CoA out of the peroxisome, as well as its possible entry into or exit from the mitochondrion, relies entirely on carnitine acetyltransferase (CAT; EC 2.3.1.7) (Sheridan *et al.*, 1990). We have therefore studied the appearance of this activity in the different cells grown *in vitro*. We consider that the results may provide useful indicators as to the likely metabolic status of this pathogenic yeast when growing in host tissues.

METHODS

Yeast and growth. *Candida albicans* C316 (a clinical isolate) was supplied by Dr Peter Chalk, Glaxo-Wellcome Research Ltd, Stevenage, UK. The culture was maintained on yeast extract/malt extract/glucose (YMG) slopes, stored at 4 °C. It was grown on a basal medium containing (g l⁻¹ distilled water): KH₂PO₄, 4.75; Na₂HPO₄, 2.1; MgSO₄·7H₂O, 0.5; yeast extract (Difco), 0.1; CaCl₂·2H₂O, 0.1; FeSO₄·6H₂O, 0.008; ZnSO₄·7H₂O, 0.001; MnSO₄·4H₂O, 0.001; CuSO₄·5H₂O, 0.005 and diammonium tartrate, 3, with the pH adjusted to 5.5. Either glucose at 56 mM, triolein (commercial trioleoylglycerol) at 2.5 mM or foetal bovine serum (Sigma) at 10% (v/v) was added as carbon source. To ensure that *C. albicans* C316 was adapted to each medium, the cells were subcultured twice on the supplemented medium. Cultures were grown at 30 °C as 100 ml batch cultures in 250 ml Erlenmeyer flasks on a gyratory shaker operating at 180 cycles min⁻¹. Cells were harvested by centrifugation (5000 g, 10 min, 4 °C), washed in distilled water and prepared for electron microscopy.

Animal models

Intraperitoneal (IP) washings and kidneys were taken from female Charles River, Harefield strain (CR-H) albino mice.

IP washings. Each of 20 mice received 0.1 ml yeast culture intraperitoneally, receiving 4×10^7 c.f.u. of *C. albicans* C316 at time zero. At 6 h post-infection, 10 animals were killed and the peritoneum washed out with 4 ml saline to remove the yeast cells. Samples were centrifuged at 3000 g for 5 min and resuspended in 1 ml saline. The samples were kept at -80 °C until required for electron microscopy.

Kidneys. Seven mice were injected intravenously with 8×10^5 blastospores of *C. albicans* C316. At 24 h post-infection, both kidneys from each mouse were removed and kept at -80 °C until required for electron microscopy. One kidney from each of two mice was homogenized in a tissue grinder for viable counts giving an average of 3.5×10^3 cells per kidney. The yeast cells were extracted from the kidney using a method based on a World Health Organization process for purification of *Mycobacterium leprae* (WHO, 1980). Chopped thawed kidney tissue was homogenized in a Potter-Elvehjem glass homogenizer with 0.14 M NaCl/0.2 M Tris base/1 mM MgSO₄ at 4 ml per g tissue. The homogenate was centrifuged (10000 g, 10 min, 4 °C), the supernatant discarded and the pellet re-homogenized,

and the process repeated. The pellet was finally resuspended in washing buffer (0.14 M NaCl/0.015 M HEPES, pH 7.2, 1 mM MgSO₄/0.1% Tween 80) and the above process repeated twice. The final pellet was resuspended in DNase buffer (0.1% Tween 80/1 mM MgSO₄/0.03 M HEPES, pH 7.2, with 4 units DNase ml⁻¹) at 4 ml per g of original tissue and incubated at 20 °C for 30 min, with stirring. The suspension was filtered through a strainer with ~0.5 mm stainless steel mesh to remove connective tissue. The suspension was centrifuged (10000 g, 10 min, 4 °C) and the resulting pellet resuspended in buffered Tween 80 (as for DNase buffer minus DNase) at 4 ml per g original tissue. This suspension was made 30% (v/v) with Percoll and was distributed to 25 ml, 27 mm diameter tubes and centrifuged (27000 g, 45 min, 4 °C), in a fixed-angle rotor. The tissue-derived debris remained as a distinct band at the top of the tube and the candidal band at the bottom. The yeast band was removed and washed in saline to remove the Percoll prior to electron microscopy.

Electron microscopy. Harvested washed yeast cells, pelleted yeast from IP washings and yeast obtained from kidney tissue were treated essentially as described by Holdsworth *et al.* (1988). The cells were fixed with KMnO₄ (1.5%, w/v) for 20 min at room temperature, washed twice in distilled water and post-stained with uranyl acetate (1%, w/v, in water) for 3 h. They were dehydrated in a graded ethanol series and embedded in Epon 812/Araldite. The embedded material was cut with diamond or glass knives on a Huxley mark I ultramicrotome. Sections were mounted on 100 mesh Formvar-coated copper grids and viewed on a JEOL JEM 100c transmission electron microscope at 80 kV. Representative sections of the whole area were recorded.

Subcellular fractionation. Spheroplasts were obtained after DeLaissé *et al.* (1981), harvested (1000 g, 10 min, 4 °C) and washed once in 1.2 M sorbitol. The spheroplasts were resuspended in 10 mM Tris/HCl (pH 7.5) containing 0.6 M sorbitol and 1 mM EDTA. The suspension was hand-homogenized in a Potter-Elvehjem glass homogenizer using a maximum of 10 passes. The cell debris (P₁) was removed by centrifugation (3000 g, 10 min, 4 °C). The supernatant (S₁) was centrifuged at 20000 g (15 min, 4 °C) to obtain the particle fraction (P₂) containing peroxisomes and mitochondria. The pellet (P₂) was resuspended in 10 mM Tris/HCl (pH 7.5) containing 0.8 M sucrose, 1 mM EDTA, 0.1% BSA and loaded on to a discontinuous density gradient of 25–55% (w/v) sucrose (Ueda *et al.*, 1982) to separate peroxisomes and mitochondria (50000 g, 3 h, 4 °C). The peroxisomal and mitochondrial bands were removed using a Pasteur pipette with the tip bent at a 90 ° angle.

Column chromatography. CAT was partially purified from crude cell extracts or isolated mitochondria and peroxisomes.

Washed cells suspended in 100 mM Tris/HCl (pH 8.0), 5 mM EDTA, 1 mM benzamidine, 0.2 mM PMSF, 1 mM DDT were disrupted by two passes through a French press (4 °C, 35 MPa). The supernatant (retrieved after centrifugation, 48000 g, 30 min, 4 °C) was taken up to 1% Triton X-100/50 mM KCl and CAT was solubilized for 18 h at 4 °C. Two ammonium sulphate fractions, at 30% and 65% saturation, were taken. The 65% pellet (18000 g, 40 min, 4 °C) was resuspended in the disruption buffer and dialysed overnight against buffer A (10 mM Tris/HCl, pH 8.0, 1 mM DTT). The desalted enzyme solution was applied to a DEAE-Sepharose-CL-6B column (3 × 9 cm) equilibrated with buffer A. After washing with buffer A, bound enzyme was eluted with a linear concentration gradient of 0.05–0.4 M KCl in buffer A. Protein was read as A₂₈₀

and the concentration gradient checked using a conductance meter. Fractions selected for enzyme studies were desalted and concentrated using Amicon concentrating cones.

Isolated peroxisomes or mitochondria were sonicated in buffer A containing 0.1% Triton X-100 for 3 min and ultracentrifuged at 139000 g, 1 h, 4 °C. Supernatants were applied to DEAE-Sephacrose-CL-6B columns (1.5 × 5 cm). CAT was eluted with a linear concentration gradient in buffer A of 0.05–0.4 M KCl.

All procedures were carried out at 0–4 °C.

Enzyme assays. CAT was assayed according to the coupled method of Chase (1969). The reaction mixture consisted of 50 mM Tris/HCl, pH 7.8, 5 mM acetylcarnitine, 3.25 mM CoASH, 5 mM EDTA, 50 mM L-malate (pH 8.0), 0.5 mM NAD⁺ (pH 6.0), 5 units citrate synthase, 4 units malate dehydrogenase and 2 mM KCN. The reaction was started with the addition of acetylcarnitine in the presence of enzyme extract. The reduction of NAD⁺ was followed at 340 nm ($\epsilon_{340} = 6.22 \times 10^6 \text{ cm}^{-1} \text{ M}^{-1}$).

Substrate specificity and apparent kinetic constants were determined using the method of Kohlhaw & Tan-Wilson (1977) at 30 °C, following the formation of free CoA via 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) at 412 nm ($\epsilon_{412} = 13.6 \times 10^6 \text{ cm}^{-1} \text{ M}^{-1}$). The reaction mixture contained 50 mM Tris/HCl, pH 8.0, 0.4 mM DTNB, 1.25 mM L-carnitine and acyl-CoA (6.25–200 µM).

2-Oxoglutarate dehydrogenase was measured at 30 °C by the method of Osmani & Scrutton (1983). The reaction mixture consisted of 100 mM K⁺-HEPES, pH 8.0, 8.4 mM 2-oxoglutarate, 2.5 mM DTT, 1 mM thiamin diphosphate, 0.5 mM NAD⁺ and 0.1 mM CoASH. The reaction was initiated by the addition of enzyme extract and followed by measuring the reduction of NAD⁺ at 340 nm.

Catalase was measured by the method of Haywood & Large (1981): 0.1 ml extract was added to 2.9 ml reagent consisting of 0.1 ml 30% (v/v) H₂O₂ in 50 ml 50 mM phosphate buffer, pH 7.0. The time taken for the A_{240} to fall from 0.45 to 0.4 was determined at 25 °C. This corresponded to decomposition of 3.45 µmol H₂O₂ in 3 ml. Activity was expressed in Sigma units: 3.45/[time (min) required].

Cytochrome c oxidase was measured by the method of Minnaert (1961). Oxidation of reduced cytochrome c was followed at 550 nm. The reaction mixture contained 65 mM phosphate buffer, pH 7.4, 1 mM EDTA, 0.006–0.07 mM cytochrome c (reduced with ascorbic acid and dialysed overnight against 50 mM phosphate buffer prior to use). Change in absorbance ($\epsilon_{550} = 22.4 \times 10^6 \text{ cm}^{-1} \text{ M}^{-1}$) was monitored at 25 °C on addition of the extract.

Protein estimations were made by the method of Bradford (1976).

RESULTS AND DISCUSSION

Internal cell structures

The basal growth medium used in this study was designed such that at 30 °C with adequate aeration, *C. albicans* C316 grew in the yeast form irrespective of the carbon source supplied (see Fig. 1a, b, c). Despite the maintenance of a regular yeast form on the different media there were some striking differences in the internal cell structure. Under the fixation protocol (KMnO₄) used here, the cytoplasm

Fig. 1. Transmission electron microscopy of *C. albicans* C316 grown at 30 °C in medium supplemented with (a) glucose, (b) triolein or (c) serum. M, mitochondrion; N, nucleus; V, vacuole; P, peroxisome; PM, plasma membrane; small arrows, distended endoplasmic reticulum; large arrow, trans-cell wall structure. Bars, 1 µm for (a) and (b); 0.5 µm for (c).

Fig. 2. Transmission electron microscopy of *C. albicans* C316 washed from the IP cavity of female CR-H mice, 6 h post-infection. M, mitochondrion; V, vacuole; Vs, vesicles; small arrow, double-membraned vesicle; large arrow, trans-cell-wall structure. Bar, 1 μ m.

of the cells grown on glucose (Fig. 1a) had a very flocculent appearance, unlike the cells grown on triolein or serum (Figs 1b and 1c, respectively.) The flocculent appearance of the cells grown on glucose made it difficult to discern the plasma membrane, which is only partially visible in the lower portion of Fig. 1(a).

The plasma membrane of the triolein-grown (Fig. 1b) and serum-grown (Fig. 1c) cells appeared to be particularly crenulated. Such invaginations of the plasmalemma are a common feature of *Candida* species and yeasts in general (Fisher *et al.*, 1982).

A proliferation of microbodies or peroxisomes could be seen in both the triolein-grown (Fig. 1b) and serum-grown cells (Fig. 1c). In both cases, intracytoplasmic membranes, tentatively identified as the endoplasmic reticulum, were visible (Fig. 1b, c). In the cell in Fig. 1(b), some of the endoplasmic reticulum seemed especially distended at one end (arrows). Much of the membranous material appeared to be associated with the periphery of the cell. The intimate association of such membranes with the periphery of the cell has previously been observed in *C. albicans* by Rajasingham & Challacombe (1988). This can be seen in the triolein-grown cells (see Fig. 7a) and most clearly in the serum-grown cells (Figs 1c, 7b).

IP conditions appeared to be favourable for growth of the yeast *in vivo*, as could be seen by the budding of the cells (Fig. 2). At 6 h post-infection, there was a proliferation of microbodies within the cell (Fig. 2), some of which appeared to lie between the plasma membrane and the cell wall (also noted by Fischer *et al.*, 1982). There was a considerable accumulation of vesicles around the vacuole. Vesicular inclusions were clearly present and some vesicles appeared to have a double membrane (small

arrow). Double-membraned vesicles were also evident in the electron micrographs of *C. albicans* presented by Anderson *et al.* (1990).

Due to clearance of the *Candida* cells from the IP cavity, *in vivo* conditions cannot be maintained for long periods within the peritoneum. However, by using an intravenous injection of a sub-lethal dose of *C. albicans* C316, a period of infection of over 24 h was maintained. Under such conditions, there were extreme changes in the subcellular organization of the cell compared to *in vitro*-cultured cells. As in the IP cells, there was an accumulation of vesicles (Fig. 3a, b). Some vesicles and large vacuolar organelles appeared to contain inclusions (arrows). The large vacuoles also contained other vesicles. Endoplasmic reticulum was again evident at the periphery of the hyphal tip (Fig. 3b). There was no evidence of the classic eukaryotic mitochondrion, although some of the large vacuolar structures had double membranes. These structures may be exaggerated examples of mitochondria with large matrix volumes. Tanaka *et al.* (1985) and Aoki *et al.* (1989) established that the mitochondria migrated to the tip of the growing hyphae, so one would expect to be able to see evidence of a mitochondrion in Fig. 3(b), but none was evident.

Vesicles were seen to accumulate around the vacuolar membrane in the cells grown *in vivo* (Figs 2 and 3). As *C. albicans* can secrete various hydrolytic enzymes (see Ross *et al.* 1990), such vesicles may be pinched off from the central vacuole and migrate to the periphery of the cell where the enzymes could be secreted.

In addition to the vacuolar vesicles, a proliferation of peroxisomes was evident in cells grown on triolein or serum or when held *in vivo* (Figs 1b, c, 2, 3a, b). Peroxisomes were identified according to the descriptions of Vamecq & Draye (1989). Peroxisomes are generally considered to be the site of β -oxidation in yeasts and fungi (Veenhuis & Harder, 1991; Ratledge, 1994). They are also the sites where amino acids, uric acid and purines are metabolized (Veenhuis & Harder, 1987; Mannaerts & Van Veldhoven, 1990).

Although the presence of peroxisomes was clearly evident in the *in vivo*-grown cells, it was not possible to recover sufficient numbers of *C. albicans* to carry out any enzymological investigation.

Peroxisomes, acetate metabolism and CAT activities

β -Oxidation of fatty acids and deamination and degradation of amino acids to acetyl-CoA increases the requirement for translocation of acetyl units between cellular compartments (see Fig. 6). In *C. albicans* grown on glucose, CAT is the main route through which acetyl-CoA is exchanged between mitochondria and the cytosol (Sheridan *et al.*, 1990). Ueda *et al.* (1982) found that in *C. tropicalis*, grown on alkanes, CAT had a dual location, being both peroxisomal and mitochondrial. This dual location has now been verified for *C. albicans* using DEAE chromatography. Fig. 4 shows a typical elution profile of

Fig. 3. Transmission electron microscopy of *C. albicans* C316 obtained from the kidneys of female CR-H mice 24 h post-infection. V, vacuole; Vs, vesicles; P, peroxisome; F, fibrillar material; HT, hyphal tip; Bars, 0.5 µm for (a), 1 µm for (b).

Fig. 4. Elution profile from DEAE-Sephacrose-CL-6B of CAT- α and β forms – obtained from *C. albicans* C316 grown on triolein (2.5 mM) at 30 °C. ●, Total CAT activity in each 1 ml fraction; ○, protein as A_{280} ; —, KCl gradient as eluant.

CAT obtained from *C. albicans* grown on triolein. Two main peaks of CAT activity were eluted, α at 0.14 M KCl and β at 0.22 M KCl, with a small peak of activity at 0.24 M KCl. When extracts of isolated mitochondria and peroxisomes were separately applied to DEAE columns, a CAT profile corresponding to α in Fig. 4 was obtained from the peroxisomes (Fig. 5a) and to β from the mitochondria (Fig. 5b). Each main peak appeared to consist of two peaks, which may represent aggregated forms of CAT (Kozulic *et al.*, 1987). Elution profiles of

Fig. 5. Elution profiles of CATs (●) obtained from isolated subcellular organelles of *C. albicans* C316 grown on triolein (2.5 mM) at 30 °C. (a) CAT (α form) obtained from peroxisomes; (b) CAT (β form) obtained from mitochondria. Total activities given per 3 ml fraction. —, KCl gradient as eluant.

CAT, consistent within the values for CAT in Table 1, were found for *C. albicans* grown on glucose, triolein or serum (not shown).

Table 1. Carbon-source-dependent changes in subcellular CAT activity

Carbon source	Enzyme*	Specific activity [$\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] [†]	
		Mitochondria	Peroxisomes
Glucose (56 mM) ($n = 2$)	CAT	0.06 ± 0.0066	0.002 ± 0.0004
	2ODDH	0.02 ± 0.0018	ND
	CCOx	0.002 ± 0.00018	0
Triolein (2.5 mM) ($n = 4$)	CAT	4.5 ± 0.29	8.8 ± 0.32
	CCOx	4.9 ± 0.22	0.03 ± 0.0012
	Catalase	740 ± 25.9	3050 ± 244
Serum (10%, v/v) ($n = 2$)	CAT	0.27 ± 0.032	0.04 ± 0.0056
	CCOx	ND	ND
	Catalase	ND	ND

* CAT, carnitine acetyltransferase; 2ODH, 2-oxoglutarate dehydrogenase; CCOx, cytochrome *c* oxidase.

[†] Enzyme activities are expressed as the mean $\pm$ range for $n = 2$, $\pm$ SEM for $n = 4$. ND, Not determined.

Table 2. Substrate specificity of CAT, derived from different sources, for acyl-CoA derivatives

Acyl-CoA derivative	Substrate specificity (%)*				
	<i>C. albicans</i> [†]		<i>C. tropicalis</i> [‡]		Human liver [§]
	Peroxisomal	Mitochondrial	Peroxisomal	Mitochondrial	
Acetyl-CoA	100 ± 2.02 (51.6)	100 ± 1.56 (21.8)	100 (88.5)	100 (113)	60
Propionyl-CoA	9.2 ± 3.52	25.7 ± 4.62	9.4	15	100 (131)
Butyryl-CoA	1.3 ± 3.7	1.2 ± 4.21	0.1	0.3	70
Octanoyl-CoA	0	0	0	0	30
Palmitoyl-CoA	0	0	0	0	0

* The results are shown as percentages of the maximum value [means $\pm$ SEM ($n = 3$)], with absolute 100% values [units (mg protein)⁻¹] given in parentheses.

[†] Results from this study, using partially purified enzymes, assayed at 30 °C.

[‡] Results from Ueda *et al.* (1982) using purified enzymes, assayed at 30 °C.

[§] Results from Bloisi *et al.* (1990) using purified enzyme, assayed at 37 °C.

The properties of the mitochondrial and peroxisomal CAT of *C. albicans* C316 grown on triolein were similar to those of *C. tropicalis* (Ueda *et al.*, 1982). As with *C. tropicalis*, the peroxisomal enzyme had a pH optimum of 7.5–7.8. However, the mitochondrial enzyme had a pH optimum of 8.0. Both enzymes were specific for acetyl-CoA, although both showed some activity with propionyl-CoA (Table 2). No activity was found with medium- or long-chain acyl-CoAs.

The apparent Michaelis constants of mitochondrial and peroxisomal CAT for acetyl-CoA or propionyl-CoA were determined from Lineweaver–Burk plots. Peroxisomal CAT showed a greater affinity for both substrates compared to mitochondrial CAT (Table 3). The mitochondrial CAT showed affinities for both substrates in keeping with those found for *C. tropicalis* (Ueda *et al.*,

1982). The peroxisomal CAT, however, showed affinities for both substrates similar to those of human liver CAT (Bloisi *et al.*, 1990), being approximately three times the affinities of peroxisomal CAT from *C. tropicalis*.

As in *C. tropicalis*, peroxisomes and associated CAT activity are inducible in *C. albicans* and depend upon which carbon source is used for growth. The internal morphological changes in *C. albicans* in response to glucose, triolein or serum can be explained in terms of carbon catabolism and acetyl-CoA translocation towards energy and building blocks (Fig. 6). When the yeast is utilizing glucose or other carbohydrates, the mitochondria predominate (Fig. 6a). There is no peroxisomal proliferation (Fig. 1a) or induction of peroxisomal CAT (Table 1). Glycolysis supplies pyruvate, which enters the mitochondrion and is then converted by pyruvate de-

Table 3. Apparent Michaelis constants of CAT from various sources for acyl-CoA derivatives.

CAT source	K_m value (μM)	
	Acetyl-CoA	Propionyl-CoA
<i>C. albicans</i> C316*		
Mitochondrial	38 (± 5)	185 (± 17.9)
Peroxisomal	14 (± 2.4)	34 (± 7.3)
<i>C. tropicalis</i> pk233†		
Mitochondrial	36	162
Peroxisomal	42	100
Human liver (40 000 g supernatant)‡	21	28

* Results from this study, using partially purified enzymes, assayed at 30 °C [means $\pm$ range ($n = 2$)].

† Results from Ueda *et al.* (1982) using purified enzymes, assayed at 30 °C.

‡ Results from Bloisi *et al.* (1990) using purified enzyme, assayed at 37 °C.

hydrogenase into acetyl-CoA. For reactions other than the tricarboxylic acid (TCA) cycle, e.g. lipid biosynthesis, acetyl-CoA must be translocated out of the mitochondrion into the cytosol. In *C. albicans*, this is via acetylcarnitine and CAT (Sheridan *et al.*, 1990) as acetyl-CoA itself is too bulky to cross the mitochondrial membrane.

When *C. albicans* was grown on triolein, there was a proliferation of peroxisomes (Fig. 1b) and an induction of peroxisomal CAT activity to twice that found in the mitochondria (Table 1). Acetyl-CoA produced by β -oxidation of acyl-CoA within the peroxisome again cannot cross the membranes because of its size and, as with the

mitochondrion, is translocated as its carnitine derivative, via the action of CAT (Fig. 6b). A portion of the acetylcarnitine will go towards sterol synthesis and other reactions (but not fatty acid biosynthesis, which is repressed under these conditions) in the cytosol and the remaining acetylcarnitine will then enter the mitochondrion, where it is converted back into acetyl-CoA by mitochondrial CAT and is then fed into the TCA cycle and also contributes to gluconeogenesis by its conversion to oxaloacetate.

Though mitochondrial CAT and peroxisomal CAT activities were 4-fold and 20-fold greater, respectively, when serum was used as carbon source rather than glucose (Table 1), the peroxisomal activity [$0.04 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] appeared low for the large peroxisomes observed in the cell (Fig. 1c). It is possible that the enzymes of the glyoxylate bypass pathway of the TCA cycle (acetyl-CoA $\rightarrow$ citrate $\rightarrow$ isocitrate $\rightarrow$ glyoxylate $\rightarrow$ malate) may play a role in *C. albicans*. Glyoxylate bypass enzymes have been located in the peroxisomes of *C. tropicalis* (Okada *et al.*, 1986) and *C. utilis* (Zwart *et al.*, 1983). Thus, peroxisomal amino acid degradation or acyl CoA β -oxidation (Fig. 6b) would supply acetyl units to the cytosol and mitochondrion via the action of CAT (peroxisomal and mitochondrial). Acetyl-CoA would also enter the glyoxylate bypass reactions of the peroxisome and lead to the formation of C_4 compounds (mainly malate and oxaloacetate), which could then be transferred either into the cytosol as the starting materials for gluconeogenesis or into the mitochondrion for the provision of energy via the reactions of the TCA cycle.

C. albicans C316 appears capable of responding to a complex nutritional environment. *In vivo*, the peroxisomal structures (Fig. 2) demonstrate the ability of this organism to degrade carbon sources such as lipids and protein which could be expected to be 'natural' substrates for an organism growing within animal tissues.

Fig. 6. Diagrammatic representations of the role of carnitine acetyltransferases (CAT) in *C. albicans* growing (a) on glucose and (b) on triolein (or host lipids) or on amino acids derived from serum. TCA, tricarboxylic acid cycle.

Cell wall structures

In addition to the marked alterations in the membranous subcellular organelles as a response to growth medium, structural changes in the cell wall were also evident.

Cells grown on triolein (Figs 1b, 7a) or serum (Figs 1c, 7b) and cells grown *in vivo* (Figs 2, 3a), especially those from the 6 h IP washings (Fig. 2), had an increased amount of the outer electron-dense fibrillar layer. This layer was not always so obvious in cells obtained from kidney tissue (Fig. 3b). (It is possible that during the recovery of these latter cells, much of the outer layer had been washed off.) The synthesis of this outer fibrillar layer has previously been associated with the nutritional status: an intercellular matrix was seen by scanning electron microscopy on the surface of *C. albicans* when grown as colonies on agar (Joshi *et al.*, 1975) and growth of *C. albicans* on various carbohydrates, particularly galactose, in liquid media induced the synthesis of extracellular polymeric material (McCourtie & Douglas, 1981; 1985).

In addition to the outer fibrillar layer of *C. albicans* C316, an electron-dense material penetrating the cell wall to the plasmalemma side was seen, with a mass of fibrillar material protruding from the exterior of the cell. These cell wall structures were seen in cells grown on either triolein or serum and *in vivo* but not in those grown on glucose (Fig. 1a). At high magnification (Fig. 7a, b), the trans-wall structures were clearly seen to consist of a cluster of distinct lines of electron-dense material penetrating the wall, each remaining a distinct entity. At the cell surface, the structures continued and protruded out of the cell wall to the exterior of the cell. The endoplasmic reticulum located at the periphery of the cell was in close proximity with these cell wall structures (Fig. 7a, b). This relationship between the endoplasmic reticulum and cell wall structures was noted by Osumi *et al.* (1975) in *C. tropicalis* pK233 grown on n-alkanes and in *C. albicans* (Tokunaga *et al.*, 1987).

The function of these trans-wall structures is not clear, although Poulain *et al.* (1989) demonstrated that they were due to the secretion of glycoproteins. The close association of endoplasmic reticulum with these structures would suggest protein production and possible pore formation. Anderson *et al.* (1990) suggested a secretory role for these trans-wall structures, with vesicles arising from the vacuole migrating through the cytosol and the pore structures to the exterior of the cell (double-membraned vesicles could be seen at the exterior of the cell). Thus, the trans-wall structures may be a route for the increased production of fibrillar material for the exterior of the cell. This material has been associated with an increased capacity of the yeast to adhere to host cell surfaces and synthetic surfaces (reviewed by Douglas, 1987). The production of an extracellular fibrillar layer (mannoprotein) by *C. albicans* is thought to be strain specific and to be more common in virulent strains than in avirulent ones (Houston & Douglas, 1989). Related fibrillar structures have been seen in *Candida* spp. grown on alkanes (Osumi *et al.*, 1975) and may be important for uptake of a hydrophobic substrate across a hydrophilic

Fig. 7. Transmission electron microscopy of the trans-cell-wall structures of *C. albicans* C316 induced by growth at 30 °C in medium supplemented with serum (a) or triolein (b). N, nucleus; M, mitochondrion; ER, endoplasmic reticulum; F, fibrillar material; large arrow, trans-cell-wall structures. Bars, 1 µm.

cell wall (see Hommel & Ratledge, 1993). It is possible the structures observed here with *C. albicans* fulfil a similar function for the uptake of fatty acids derived either from triolein or from the host lipids.

Finally, yeast mitochondria have been found to exert control over the cell-surface complex, influencing agglutination, tolerance to drugs, utilization of sugars and plasma membrane proteins (Prasad, 1985, and references therein). Under O₂ limitation, or in the presence of excess reducing equivalents due to oxidative biochemical pro-

cesses, one would expect respiratory changes and, thus, possible alterations in the mitochondria. These changes may be manifest through cell wall changes in *C. albicans* C316.

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