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Export of steryl esters from lipid particles and release of free sterols in the yeast, *Saccharomyces cerevisiae*

Regina Leber, Erwin Zinser, Claudia Hrastnik, Fritz Paltauf, Günther Daum *

Institut für Biochemie und Lebensmittelchemie, Technische Universität Graz, Petersgasse 12 / 2, A-8010 Graz, Austria

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Abstract

Fatty acyl esters of the yeast specific sterol, ergosterol, are exclusively stored in lipid particles. Under conditions of sterol deficiency, e.g., in the presence of terbinafine, an inhibitor of fungal squalene epoxidase, steryl esters are hydrolyzed, and sterols are set free for membrane formation. Lipid particles do not contain steryl-ester hydrolase activity themselves; the highest specific activity of this enzyme is found in the plasma membrane. Therefore, steryl esters have to be exported from lipid particles to their site of hydrolytic cleavage. This process of translocation and metabolic conversion was studied in vivo. Addition of nocodazole to terbinafine-treated cells did not disturb the mobilization of steryl esters, indicating that this process is not mediated by microtubuli-dependent vesicle flux. Under the influence of inhibitors of cellular energy production (azide and fluoride) and protein biosynthesis (cycloheximide) mobilization of steryl esters came to an halt. These results support the view that ongoing membrane proliferation may be a driving force for the release of sterols from steryl esters of lipid particles.

Keywords: Lipid particle; Sterol transport; Steryl ester; Steryl-ester hydrolase; Plasma membrane; Terbinafine; (*S. cerevisiae*)

1. Introduction

Sterols are integral components of eukaryotic membranes. In yeast cells similar to higher eukaryotes the plasma membrane exhibits the highest sterol to protein and sterol to phospholipid ratio of all subcellular membranes [1,2]. The site of sterol synthesis has been reported to be the microsomal fraction [3] with specific contributions of the so-called lipid particles [2,4]. In the endoplasmic reticulum sterols can be esterified with long-chain fatty acids, and steryl esters formed are stored in lipid particles together with triacylglycerols [1,2,4,5]. Depending on the growth stage steryl esters are metabolically interconvertible with free sterols [6,7]. The enzyme catalyzing the hydrolytic cleavage of steryl esters, steryl-ester hydrolase, was detected at highest specific activity in the plasma membrane and protein secretory vesicles [2]. The latter location was found in temperature-sensitive yeast secretory mutants, which are blocked at a late stage of the protein secretory pathway. In these mutants proteins of the plasma membrane, among them steryl-ester hydrolase, accumulate in secretory vesicles.

The spatial separation of enzymes involved in the synthesis of sterols and steryl esters, and in the hydrolytic cleavage of the latter components necessitates several steps of intracellular sterol transport. Mechanisms proposed for sterol translocation are monomer transport with or without the aid of sterol carrier proteins, or vesicles flow with or without an involvement of the protein secretory machinery (for reviews see Refs. [8,9]). While there is little proof for a participation of sterol carrier proteins in the intracellular movement of sterols in vivo, increasing evidence points to vesicle flow as a possible mechanism. It is an open question whether protein secretory vesicles or other types of intracellular vesicles govern this process. Enrichment of the yeast specific sterol, ergosterol, in protein secretory vesicles isolated from the temperature-sensitive yeast secretory mutant *Saccharomyces cerevisiae sec1* [2] favors the view of the participation of the protein secretory machinery in sterol transport of yeast. On the other hand, sterol transport to the plasma membrane of mammalian cells was found to be unaffected by monensin and brefeldin A, drugs which are known to inhibit the secretion of proteins [10,11].

* Corresponding author. Fax: +43 316 810599.

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Lipid particles of the yeast known as storage granula for triacylglycerols and steryl esters seem to be involved in sterol metabolism and transport. Recent observations in our laboratory demonstrated that at least one enzyme involved in sterol biosynthesis, sterol Δ-24 methyltransferase, is a component of lipid particles [4]. Sterols are obviously synthesized in a concerted action of enzymes located on the surface of the endoplasmic reticulum and lipid particles. Nothing is known about the cellular mechanism(s) leading to the deposition of steryl esters in lipid particles, nor about their export from this compartment during the mobilization of this sterol source. In order to characterize the latter process we designed an assay in vivo using terbinafine, an inhibitor of squalene epoxidase [12]. Terbinafine causes sterol deficiency, which in turn leads to a mobilization of steryl esters from lipid particles. Cellular requirements for this process were tested using specific inhibitors of energy production, protein biosynthesis, and cytoskeleton integrity.

2. Materials and methods

2.1. Yeast strains, culture conditions and organelle preparations

The wild-type yeast strain *Saccharomyces cerevisiae* X2180-1A (**a**, *SUC2*, *mal*, *gal2*, *CUP1*) was cultivated in 500 ml or 2 l Erlenmeyer flasks at 30°C in a rotary shaker with vigorous aeration. For the preparation of lipid particles and plasma membrane cells were pregrown in rich medium (YPD; 3% glucose, 1% yeast extract and 2% peptone) overnight to the exponential growth phase. Then cells were transferred to fresh sterol-free medium [13] buffered with citrate at pH 6.0. Terbinafine dissolved in ethanol was added at a final concentration of 30 μg/ml, and cells were grown for 4 h at 30°C. Control cells were incubated for the same period in the presence of the corresponding amount of ethanol, but without terbinafine. Lipid particles [4], plasma membrane [14], vacuoles [4], and microsomes [1] were prepared by published procedures. Isolated fractions were characterized by SDS-PAGE and Western blotting (see below), and exhibited the same purity as described previously [1,2,4].

2.2. Steryl ester mobilization assay in vivo

Yeast cells were pregrown in YPD medium for 16 h and transferred to sterol-free medium as described above. The culture was divided into two portions, and one half was treated with terbinafine (added as ethanolic solution) at a final concentration of 30 μg/ml, the other half remained untreated as a control, but received the same volume of ethanol. Growth of yeast cells was determined by measuring the optical density with a Hitachi U1100 UV/VIS Spectrophotometer at a wavelength of 600 nm.

At time-points indicated aliquots of the culture (15 ml) were withdrawn, and cells were harvested by centrifugation on a table top centrifuge. After shock-freezing in liquid nitrogen 5 ml chloroform/methanol (2:1; v/v) were added, and cells were disintegrated by vigorous shaking in the presence of 1 ml glass beads (diameter 0.3–0.4 mm) for 10 min at 4°C on an EVAPO-MIX (Buchler Instruments, Fort Lee, NJ, USA). Cellular lipids were extracted as described by Folch et al. [15]. $MgCl_2$ (1 ml; 0.034%) was added, and samples were shaken for another 30 min at 4°C. After centrifugation for 5 min on a table top centrifuge the aqueous phase was removed, and the organic phase was washed once with 1 ml methanol/water/chloroform (48:47:3; per vol.). Again the aqueous phase was removed, and the organic phase was saved. The sediment of glass beads formed during the previous steps was reextracted once with chloroform/methanol (2:1; v/v). Organic phases were combined, taken to dryness, and lipids were dissolved in 500 μl chloroform/methanol (2:1; v/v).

When yeast cells were treated with nocodazole (Sigma), the drug was dissolved in DMSO and added to the culture medium at a concentration of 15 μg/ml 2.5 h prior to the shift to sterol-free medium. Nocodazole-treated cells were arrested as large budded cells as described in the literature [16]. Sterol-free media contained either nocodazole (15 μg/ml), nocodazole plus terbinafine (same concentration as described above), or equivalents of DMSO and/or ethanol (solvents) as a control. Samples were withdrawn, and lipids were extracted as described above.

In order to test the effect of energy depletion in combination with the inhibition of sterol biosynthesis by terbinafine, wild-type yeast cells pregrown in rich medium (YPD) for 16 h were transferred to sterol-free medium, and cultures were divided into three portions. One culture was further incubated in the presence of terbinafine (30 μg/ml), the others in the absence of the inhibitor of sterol biosynthesis. After 30 min azide and fluoride (5 mM final concentration, each) were added to terbinafine-treated cells and to one of the untreated cultures. The third culture, which served as a control, was devoid of any inhibitor.

A similar protocol was used for the incubation of cells with cycloheximide in combination with terbinafine. One culture contained terbinafine (30 μg/ml) and cycloheximide (5 μg/ml), the second cycloheximide (5 μg/ml), and the third no inhibitors at all.

2.3. Labelling of the cellular lipids with [^{14}C]acetate in the presence or absence of terbinafine

Wild-type yeast cells were grown overnight in YPD medium and transferred to fresh sterol-free medium. The culture was divided into two portions (100 ml; OD 2 each), and terbinafine (final concentration 30 μg/ml) was added to one of them. Then cells were labeled in the presence of [^{14}C]acetate (12.5 μCi; 3.7 μmol). Aliquots of the cultures

were withdrawn at time-points indicated, and cells were harvested by centrifugation. Cellular lipids were extracted with chloroform/methanol (2:1; v/v) by the method of Folch et al. [15] after shock-freezing of cells as described above.

2.4. Lipid analyses

Neutral lipids were separated by thin-layer chromatography using silica gel 60 plates (Merck, Darmstadt, Germany). A two-step development system was employed. Solvent I contained light petroleum/diethyl ether (1:1, v/v). The chromatogram was developed till the solvent front reached one third of the height on the thin-layer plate. After drying the plate carefully, solvent II consisting of light petroleum/diethyl ether (98:2; v/v) was used for development in the same direction to the top of the thin-layer plate. This chromatographic system allows the separation of free sterols, terbinafine, steryl esters, squalene and triacylglycerols. Concentrations of ergosterol and ergosteryl ester were determined after chromatographic separation by direct densitometry on thin-layer plates at 275 nm using a Shimadzu CS 930 thin-layer chromatography scanner with ergosterol as a standard. Squalene was visualized after separation on thin-layer plates by dipping the plates into conc. H_2SO_4/water/methanol (1:1:18; by vol.) and heating at 120°C for 15 min. Densitometric scanning was carried out at 465 nm using commercially available squalene as a standard. Triacylglycerols were visualized by dipping thin-layer plates into a solution containing 0.4 g $MnCl_2$, 60 ml water, 60 ml methanol and 4 ml H_2SO_4, and heating at 120°C for 15 min. Quantitation of triacylglycerols was carried out by densitometric scanning at 400 nm with triolein as a standard.

Labeled neutral lipids were separated on silica gel 60 thin-layer chromatography plates employing the two-step development system described above. In order to separate diacylglycerols from free sterols a developing solvent containing toluene/diethyl ether/ethyl acetate/25% ammonia (80:10:10:0.2; by vol.) was used. Lipids were visualized on thin-layer plates after exposure to iodine vapor, scraped off, and subjected to liquid scintillation counting using LSC Safety Cocktail (Packard) with 5% water.

Phospholipids were quantitated as described by Broekhuyse [17].

2.5. Protein quantitation, SDS-polyacrylamide gel electrophoresis, and Western blot analysis

Proteins were quantitated by the method of Lowry et al. [18] using bovine serum albumin as a standard. Lipid particles (20 μg protein) had to be delipidated by extraction with diethyl ether (2×0.5 ml) prior to the colorimetric assay. Proteins were routinely precipitated with 10% trichloroacetic acid and solubilized in 0.1% SDS-0.1 M NaOH for quantitation.

For SDS-polyacrylamide gel electrophoresis of their proteins, lipid particles were delipidated with diethyl ether as described above. Protein (20 μg) was precipitated with 10% trichloroacetic acid, washed with distilled water at 0°C, and dissolved in dissociation buffer containing 6% SDS. Protein bands on the gel were visualized with Coomassie Brilliant Blue R.

Immunological characterization of subcellular fractions was carried out by standard techniques [19] after separating proteins on 12.5% SDS polyacrylamide gels and transferring to nitrocellulose filters (Hybond™-C, Amersham). Proteins were detected by ELISA using rabbit antibodies against the respective antigens, and peroxidase-conjugated goat-anti-rabbit secondary antibodies. Antisera against yeast plasma membrane ATPase and yeast carboxypeptidase Y were gifts of R. Serrano, Valencia, Spain, and D. Wolf, Stuttgart, Germany, respectively.

3. Results

Exclusive storage of steryl esters in lipid particles on one hand, and localization of steryl-ester hydrolase at highest specific activity in the plasma membrane and in secretory vesicles [2] of the yeast, *Saccharomyces cerevisiae*, on the other hand, raised the question as to pathway(s) and mechanism(s) of the migration of these lipids to the cell periphery. We designed an assay to study export of steryl esters from lipid particles and hydrolysis in vivo, and made use of terbinafine, an inhibitor of fungal squalene epoxidase, to cause sterol deficiency. Synthesis of sterols de novo and, as a consequence, of steryl esters was inhibited, when yeast cells were incubated in sterol-free medium at pH 6.0 with 30 μg/ml terbinafine [12]. Treatment of cells with terbinafine resulted in a dramatic reduction of the rate of incorporation of [^{14}C]acetate into sterols and steryl esters (Fig. 1C) to less than 5% of the control (Fig. 1B), whereas an accumulation of the label in squalene was observed (Fig. 1C). These data demonstrate, that under conditions chosen for this in vivo assay yeast cells could not synthesize sterols any more, although cellular growth continued for several hours at a reduced rate (Fig. 1A). Inhibition of de novo sterol synthesis was a prerequisite for measuring the mobilization of pre-existing cellular pools of steryl esters. Enzyme assays in vitro (data not shown) demonstrated that terbinafine did not influence steryl-ester hydrolase.

When wild-type yeast cells were incubated in the presence of 30 μg/ml terbinafine, the cellular level of ergosteryl esters dropped to zero within 2 h (Fig. 2C), indicating that the sterol moiety of ergosteryl esters was set free under conditions of sterol depletion. The cellular amount of free ergosterol decreased to approx. 60% of the starting level within 4 h, and a dramatic accumulation of squalene was observed due to the inhibitory effect of terbinafine on squalene epoxidase. In control cells (Fig.

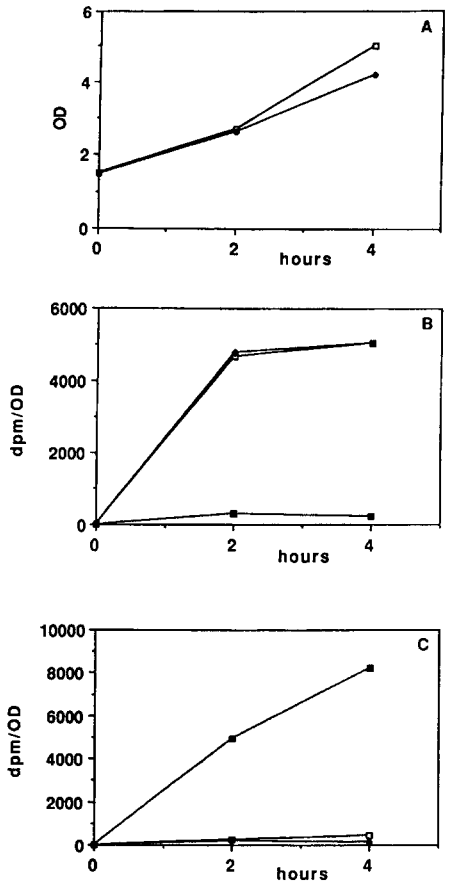

Fig. 1. Labelling of wild-type yeast cells (*Saccharomyces cerevisiae* X2180-1A) with [^{14}C]acetate. Yeast cells were grown overnight in YPD-medium, transferred to sterol-free medium, and incubated in the presence (30 μg/ml) or absence of terbinafine. Labelling conditions with [^{14}C]acetate are described under Materials and methods. At time-points indicated aliquots of the culture were withdrawn, and lipids were extracted and analyzed. (A) Growth curves of yeast cells grown in the presence (♦) or absence (□) of terbinafine. (B) Distribution of ^{14}C-label in ergosterol (□), ergosteryl esters (♦), and squalene (■) of cells grown in the absence of terbinafine. (C) Distribution of ^{14}C-label in ergosterol (□), ergosteryl esters (♦), and squalene (■) of cells grown in the presence of terbinafine.

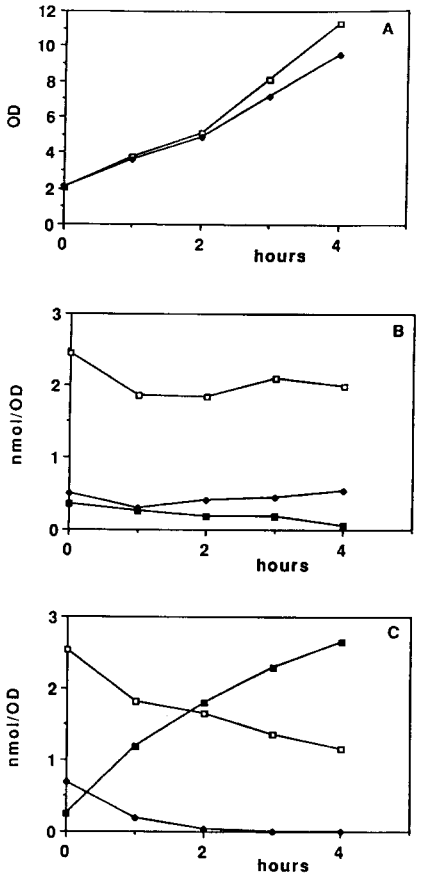

Fig. 2. Mobilization of steryl esters under conditions of sterol depletion. Cells were pregrown in YPD-medium overnight, transferred to sterol-free medium, and incubated in the presence (30 μg/ml) or absence of terbinafine. At time-points indicated cells were harvested, lipids were extracted and analyzed by optical scanning on thin-layer plates as outlined in Materials and methods. (A) Growth curves of yeast cells grown in the presence (♦) or absence (□) of terbinafine. (B) Amount of ergosterol (□), ergosteryl esters (♦), and squalene (■) per OD of cells grown in the absence of terbinafine. (C) Amount of ergosterol (□), ergosteryl esters (♦), and squalene (■) per OD of cells grown in the presence of terbinafine.

2B) concentrations of free ergosterol, ergosteryl esters and squalene remained fairly constant over the whole period of the experiment. The fact that cellular growth (Fig. 2A) was only slightly reduced when the concentration of steryl esters had dropped to zero demonstrates, that this lipid is not essential for yeast cells. In contrast a minimum level of free sterols (approx. 25% of normal) is absolutely required for yeast cell viability [20]. This level was not reached under conditions chosen for the experiment shown in Fig. 2.

Detailed analysis of subcellular fractions (Table 1) demonstrated that under conditions of sterol deficiency, i.e., in the presence of the inhibitor terbinafine, steryl esters of lipid particles were completely used up. The level of triacylglycerols in lipid particles, on the other hand, was only slightly decreased, which is consistent with the view that a selective mobilization of steryl esters, but not a 'bulk mobilization' of lipid particles, occurred. Interestingly squalene, the metabolic intermediate accumulating in

Table 1
Lipid composition of lipid particles and plasma membrane of wild-type yeast cells grown in the absence or presence of terbinafine

	Lipid composition (mg/mg protein)			
	lipid particles		plasma membrane	
	−terbinafine	+terbinafine	−terbinafine	+terbinafine
Ergosterol	0.06	n.d.	0.13	0.09
Ergosteryl esters	2.6	n.d.	traces	n.d.
Squalene	1.1	4.5	traces	traces
Triacylglycerols	19.6	16.1	–	–
Phospholipids	–	–	0.251	0.255

Yeast cells were grown for 4 h in the presence or absence of terbinafine as outlined in Materials and methods. Mean values of three independent experiments are shown. n.d., not detectable; –, not determined.

terbinafine-treated cells, was highly enriched in lipid particles. Synthesis of squalene has been reported to be a microsomal process (see Ref. [3]), and its translocation to lipid storage particles might be the consequence of the overproduction. The plasma membrane of terbinafine-treated yeast cells exhibited a lower concentration of free ergosterol than the plasma membrane of control cells. This decrease to 60% of the control reflected the overall situation in whole cells (see Fig. 2C). Neither steryl esters nor squalene were found at significant amounts in the plasma membrane of terbinafine-treated cells.

Where does the hydrolysis of steryl esters occur in yeast cells treated with terbinafine? In untreated cells the highest specific activity of steryl-ester hydrolase (0.35 nmol/min per mg) was attributed to the plasma membrane; only minor activities were found in other organelles (see Ref. [2]). Lipid particles are absolutely devoid of steryl-ester

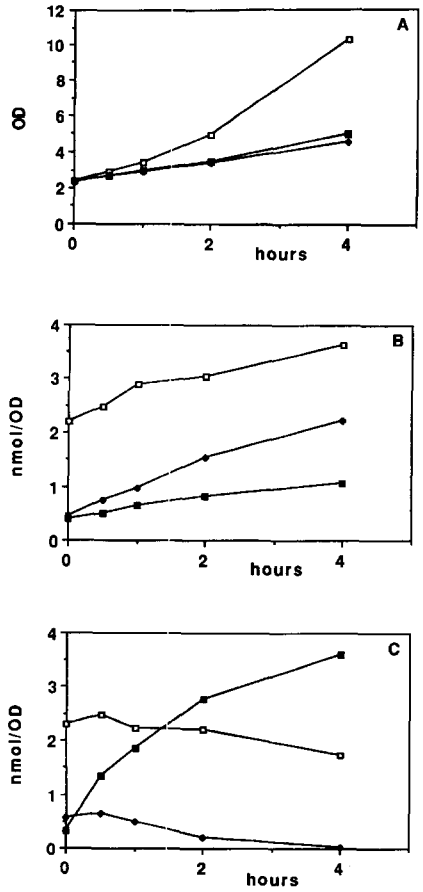

Fig. 3. The microtubuli disrupting agent nocodazole does not inhibit the mobilization of steryl esters. Cells were pregrown on YPD-medium. 2.5 h prior to the shift to sterol-free medium 15 μg/ml nocodazole was added. The sterol-free medium contained the same concentration of nocodazole throughout the whole experiment. (A) Growth curves of yeast cells grown in the presence of nocodazole (♦), of terbinafine and nocodazole (■), or in the absence of both drugs (□). (B) Amount of ergosterol (□), ergosteryl esters (♦), and squalene (■) per OD of cells grown in the presence of nocodazole, but in the absence of terbinafine. (C) Amount of ergosterol (□), ergosteryl esters (♦), and squalene (■) per OD of cells grown in the presence of nocodazole and terbinafine.

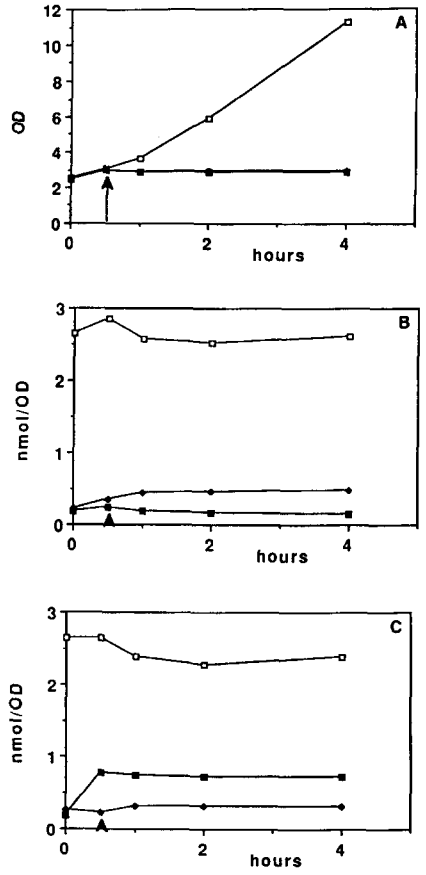

Fig. 4. Energy dependence of the mobilization of steryl esters. Cells were pregrown on YPD-medium and shifted to sterol-free medium. After 30 min of incubation (indicated by the arrow) azide and fluoride (final concentration 5 mM each) were added. (A) Growth curves of yeast cells grown in the presence of azide and fluoride (♦), of terbinafine, azide and fluoride (■), or in the absence of all inhibitors (□). (B) Amount of ergosterol (□), ergosteryl esters (♦), and squalene (■) per OD of cells incubated in the presence of azide and fluoride, but in the absence of terbinafine. (C) Amount of ergosterol (□), ergosteryl esters (♦), and squalene (■) per OD of cells grown in the presence of azide, fluoride and terbinafine.

hydrolase activity. Similar results were obtained with subcellular fractions of terbinafine-treated cells. Again preparations of the plasma membrane exhibited the highest specific activity of steryl-ester hydrolase (0.30 nmol/min per mg), and lipid particles completely lacked this enzyme activity. The fact that lipid particles are physically associated with vacuoles in vivo as observed by microscopic inspection (E. Zinser, unpublished observation) led us to speculate that vacuoles, like lysosomes in mammalian cells (for a review see Ref. [21]), might be involved in hydrolytic cleavage and mobilization of steryl esters. However, steryl-ester hydrolase could never be measured at a significant activity in highly purified preparations of yeast vacuoles.

The assay system in vivo described above allowed us to test cellular requirements for the acquisition of sterols from steryl esters, which is linked to the export of the

latter component from lipid particles. We first asked the question whether or not mobilization of steryl esters from lipid particles depends on the existence of an intact cytoskeleton. We employed nocodazole (15 μg/ml) to disrupt microtubuli of yeast cells during a preincubation of 2.5 h. Cellular growth of nocodazole-treated yeast was largely inhibited (Fig. 3A). During continued incubation with nocodazole an increase in the amounts of ergosteryl esters, free ergosterol and squalene was observed (Fig. 3B). The reason for this effect seems to be the ongoing synthesis of sterols while the growth rate was strongly reduced. When terbinafine was added to cells pretreated with nocodazole (Fig. 3C) the concentration of ergosteryl esters reached the zero level after 4 h. The slower rate of mobilization as compared to the control without nocodazole (see Fig. 2C) was probably due to the reduced growth rate.

In order to test the energy requirement of the transport-linked mobilization of ergosteryl ester from lipid particles, yeast cells treated with azide and fluoride were incubated with or without terbinafine (Fig. 4). When azide and fluoride were added to the culture medium, cells stopped growing after 30 min (Fig. 4A). A slight increase in the concentration of steryl esters and a minor decrease of free sterols was observed (Fig. 4B). Internal pools of activated fatty acids might be utilized for steryl ester biosynthesis at this stage. Then the concentration of all lipids remained constant, indicating that neither synthesis of sterols and steryl esters, nor interconversion of these two lipid species occurred. Levels of lipids were also unchanged when terbinafine was present in the culture medium in addition to azide and fluoride (Fig. 4C).

The view that a linkage between mobilization of steryl esters from lipid particles and membrane proliferation exists was supported by an experiment using cycloheximide (5 μg/ml) as an inhibitor in addition to terbinafine. Cycloheximide, an inhibitor of protein synthesis in eukaryotes, was added to the culture medium after cells had grown for 30 min in the presence (Fig. 5C) or absence (Fig. 5B) of terbinafine. Cycloheximide dramatically reduced the growth rate of yeast cells (Fig. 5A). In the absence of terbinafine cycloheximide caused an increase of the concentration of steryl esters and squalene, but not of free sterols (Fig. 5B). When cycloheximide was used in combination with terbinafine, the cellular content of steryl esters continued to decrease slightly for 30 min after the addition of cycloheximide, but then remained constant (Fig. 5C).

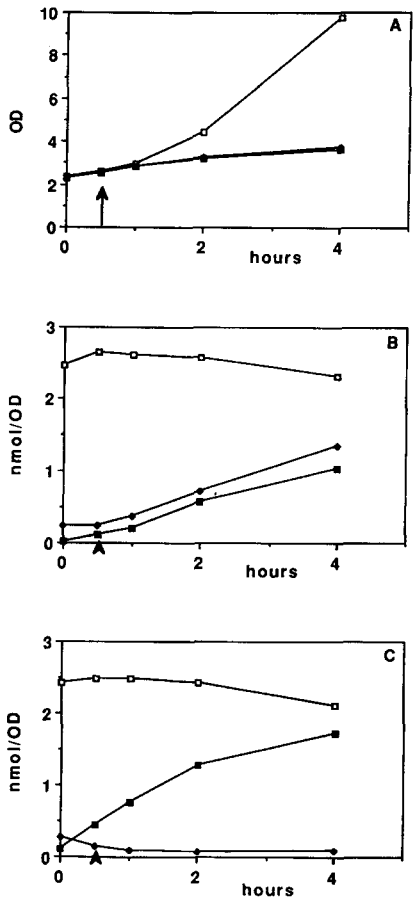

Fig. 5. Mobilization of steryl esters in yeast cells treated with cycloheximide. Cells were pregrown on YPD-medium and shifted to sterol-free medium. After 30 min of incubation (indicated by the arrow) cycloheximide (final concentration 5 μg/ml) was added. (A) Growth curves of yeast cells grown in the presence of cycloheximide (♦), of terbinafine and cycloheximide (■), or in the absence of all inhibitors (□). (B) Amount of ergosterol (□), ergosteryl esters (♦), and squalene (■) per OD of cells incubated in the presence of cycloheximide, but in the absence of terbinafine. (C) Amount of ergosterol (□), ergosteryl esters (♦), and squalene (■) per OD of cells grown in the presence of cycloheximide and terbinafine.

4. Discussion

In the present paper we propose the involvement of lipid particles, the site of storage of sterols esterified with long-chain fatty acids, in the intracellular transport of sterols, based on synthesis and hydrolysis of steryl esters. In the yeast, *Saccharomyces cerevisiae*, steryl esters and free sterols are interconvertible depending on the growth phase. The concentration of steryl esters is high in the stationary phase, but relatively low in the lag and exponential growth phase [6,7]. Under sterol depletion caused by incubation of yeast cells with terbinafine, steryl esters were mobilized very rapidly even in the exponential growth phase (see Fig. 2). The reason for this mobilization is obviously a demand of free sterols for membrane formation, mainly of the plasma membrane. Lipid particles isolated from cells 4 h after the addition of terbinafine were completely devoid of steryl esters, whereas the plasma membrane still contained sterols at a level sufficient for cell viability (see Table 1). Levels of free sterols and steryl esters in other organelles, e.g., vacuoles or microsomes were not affected by the treatment with terbinafine.

Experiments with deenergized yeast cells (see Fig. 4) suggest that either transport of steryl esters from lipid

particles to their site of hydrolysis (plasma membrane) is energy dependent, or that the requirement of free sterols coupled to membrane proliferation, especially to that of the plasma membrane, is a driving force for steryl ester mobilization. When yeast cells were poisoned with cycloheximide membrane proliferation stopped, but cellular energy levels were maintained. Under these conditions hydrolysis of steryl esters came to an halt (see Fig. 5) suggesting that transport of steryl esters to the site of hydrolysis and ongoing membrane proliferation may be linked processes. This view is supported by the finding that yeast cells accumulate steryl esters in lipid particles in the stationary growth phase, because there is practically no membrane proliferation at this stage. As a consequence the pathway of steryl ester mobilization from lipid particles might be inactivated, and sterols still produced are stored in the form of steryl esters in lipid particles. Further, although indirect support to this hypothesis comes from the observation, that all attempts failed to reconstitute migration of steryl esters from lipid particles to the plasma membrane and subsequent hydrolysis in vitro, i.e., under conditions when cell proliferation does not occur (E. Zinser, unpublished results). Plasma membrane proliferation as a driving force of sterol acquisition was also suggested by Billheimer and Reinhart [8] for mammalian cells.

Export of steryl esters from lipid particles does not depend on the existence of intact microtubuli as demonstrated by experiments using the microtubuli disrupting agent nocodazole (see Fig. 3). Energy driven vesicle flux, e.g., flow of protein secretory vesicles [22] as a mechanism of steryl ester transport to the cell periphery is therefore unlikely. At present we can only speculate about the true mechanism(s) of this translocation process. Flux of specialized vesicles described previously for the transport of phospholipids to the plasma membrane of *Dictyostelium discoideum*, which are different from protein secretory vesicles [23], could be involved. Alternatively lipid particles might migrate directly to the site of steryl ester hydrolysis. During membrane contact, e.g., with the plasma membrane, the subcellular location with the highest specific activity of steryl-ester hydrolase and the maximum requirement of free sterols, an intermediate complex might be formed, which would allow release and subsequent hydrolysis of steryl esters. A similar mechanism was proposed by Reinhart [9] for the translocation of cholesterol esters from lipoproteins to the plasma membrane of mammalian cells. Interaction of yeast lipid particles with other organelles, e.g., the plasma membrane, which harbors steryl-ester hydrolase activity, cannot be an unspecific event, and might be triggered by organellar factors. Surface proteins of lipid particles [4] might mediate this process. Experiments are on the way to characterize lipid particle proteins in order to obtain more insight into the molecular basis of interorganelle translocation of sterols.

More than 50% of sterols esterified with fatty acids and present in lipid particles are different from ergosterol, namely zymosterol, episterol, fecosterol and other intermediates of sterol biosynthesis [2]. Free sterols of the plasma membrane, on the other hand, comprise 95% ergosterol, also in cells treated with terbinafine. Fatty acyl esters of sterol intermediates are obviously not transported directly to the plasma membrane, hydrolyzed and inserted without prior metabolic conversion. Since conversion of precursor sterols to ergosterol has never been shown to occur at the stage of steryl esters, hydrolytic cleavage of the latter sterol derivatives should precede the formation of the final product, ergosterol. In that case, conversion of precursors to ergosterol should either be catalyzed by enzymes located on the surface of lipid particles, or lipid particles would have to interact with internal membranes which harbor enzymes required for the respective metabolic steps.

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