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Sterol Composition of Yeast Organelle Membranes and Subcellular Distribution of Enzymes Involved in Sterol Metabolism

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Organelles of the yeast *Saccharomyces cerevisiae* were isolated and analyzed for sterol composition and the activity of three enzymes involved in sterol metabolism. The plasma membrane and secretory vesicles, the fractions with the highest sterol contents, contain ergosterol as the major sterol. In other subcellular membranes, which exhibit lower sterol contents, intermediates of the sterol biosynthetic pathway were found at higher percentages. Lipid particles contain, in addition to ergosterol, large amounts of zymosterol, fecosterol, and episterol. These sterols are present esterified with long-chain fatty acids in this subcellular compartment, which also harbors practically all of the triacylglycerols present in the cell but very little phospholipids and proteins. Sterol Δ^{24}-methyltransferase, an enzyme that catalyzes one of the late steps in sterol biosynthesis, was localized almost exclusively in lipid particles. Steryl ester formation is a microsomal process, whereas steryl ester hydrolysis occurs in the plasma membrane and in secretory vesicles. The fact that synthesis, storage, and hydrolysis of steryl esters occur in different subcellular compartments gives rise to the view that ergosteryl esters of lipid particles might serve as intermediates for the supply of ergosterol from internal membranes to the plasma membrane.

Lipid transport in eukaryotic cells is an essential process, because synthesis of lipids is restricted to certain organelles, whereas lipids are required as constitutive components of all subcellular membranes (3, 37). Lipid migration must be efficiently regulated, because lipids are not randomly distributed among subcellular membranes. In fact, certain lipids are characteristic for specific membranes, e.g., cardiolipin for the inner mitochondrial membrane (6) and sterols (15, 41) and sphingolipids (15, 24) for the plasma membrane. Possible mechanisms of lipid transport are spontaneous or protein-catalyzed transfer of lipid monomers between membranes, vesicle flow, and membrane contact and fusion (3).

Sterols are essential components of the eukaryotic plasma membrane. The mechanism of their transport from internal membranes, where they are synthesized, to the periphery of the cell is still obscure. Vesicle flow as a possible mechanism seems very likely, but the vesicles involved need not be identical to protein secretory vesicles (36). Sterol carrier proteins, which have been shown to stimulate translocation of sterols in vitro, have not been proven to catalyze this process in vivo (1).

We have chosen the yeast *Saccharomyces cerevisiae* as a model cell to study intracellular transport of sterols. The yeast-specific sterol ergosterol is structurally and functionally related to sterols found in higher eukaryotes. Under conditions in which yeast cells cannot produce their own ergosterol, e.g., under anaerobiosis, in auxotrophic mutants, or in the presence of inhibitors of sterol biosynthesis, addition of ergosterol to the growth medium and uptake into cells are essential for cellular growth and proliferation (27, 28).

The plasma membrane of *S. cerevisiae*, with its extremely high ergosterol-phospholipid ratio of 3.3 (mol/mol) is the major subcellular location of free sterol (41) in *S. cerevisiae*. The fact that secretory vesicles isolated from the *S. cerevisiae sec1* mutant (12) are the organelle with the next highest content of free sterols might indicate that secretory vesicles can contribute to the supply of sterols to the cell periphery. Esterified sterols are found more or less exclusively in the so-called lipid particle fraction, which, in addition to steryl esters, contains large amounts of triacylglycerols (5).

In the present study, we performed detailed analyses of sterols in yeast subcellular membranes to define more clearly the destinations of sterols within the cell. We then analyzed isolated organelle membranes for the activity of three enzymes involved in sterol metabolism. Sterol Δ^{24}-methyltransferase is one of the late enzymes in ergosterol biosynthesis and defines a possible starting point of intracellular sterol transport. Steryl ester synthase and steryl ester hydrolase are key enzymes involved in the homeostasis of free ergosterol in yeast cells (16). The impact of the subcellular distribution of these enzymes on possible mechanisms of sterol transfer in *S. cerevisiae* is discussed. A possible role of lipid particles in sterol translocation is proposed.

MATERIALS AND METHODS

Yeast strains and culture conditions. *S. cerevisiae* X2180-1A (a *SUC2 mal gal2 CUP1*), *S. cerevisiae* D273-10B (ATCC 25657; α), and secretory mutant *S. cerevisiae sec1* (provided by R. Schekman) were used throughout this study. *S. cerevisiae* X2180-1A and *sec1* were pregrown on YPD medium (1% yeast extract, 2% peptone, 2% glucose), and *S. cerevisiae* D273-10B was grown on YPLac (2% lactate) (7) for 2 days and inoculated into fresh media at a dilution rate of 1:2,500 (YPD medium) or 1:500 (YPLac medium). Incubations were carried out to the mid-exponential growth phase in 2-liter flasks (500 ml of media) at 30°C (wild-type cells) or 24°C (*sec1* cells) on a rotary shaker with vigorous

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aeration. To induce the secretion block in *S. cerevisiae sec1*, cells were shifted to the nonpermissive temperature of 37°C for 2 h prior to harvesting.

Cell fractionation. Plasma membrane was prepared by the method of Serrano (31). To obtain microsomes and cytosol from the same preparation, the 20,000 × *g* supernatant was centrifuged for 1 h at 100,000 × *g* (T-865 rotor; Sorvall); the clear supernatant is the cytosolic fraction. The resulting pellet was suspended in 10 mM Tris Cl, pH 7.4, with a Dounce homogenizer (Braun) with a tightly fitting pestle. The suspension was then centrifuged at 20,000 × *g* for 20 min to remove most of the remaining contaminating plasma membrane and mitochondria. The resulting supernatant was centrifuged at 100,000 × *g* for 1 h, yielding a colorless, opaque pellet consisting mostly of microsomal membranes. Different microsomal fractions were isolated as described elsewhere (41). Nuclei were prepared by the procedure of Hurt et al. (13), and mitochondria and subfractions of mitochondria were isolated by the method of Daum et al. (7). Secretory vesicles were obtained from secretory mutant *S. cerevisiae sec1* after a shift to the restrictive temperature (37°C) for 2 h on low-glucose YPD medium (0.1% glucose) to impose the secretion block and to induce the marker enzyme invertase (38). Vacuoles were isolated by the method of Uchida et al. (35) with several modifications. The original procedure does not allow sufficient separation of vacuoles and adhering lipid particles. Thus, the protocol was modified to separate and enrich both lipid particles and vacuolar membranes to a large extent. After the last step of flotation (35), the crude vacuolar fraction containing lipid particles was suspended in 5 mM MES-Tris (pH 6.8)–0.6 M sorbitol with a loosely fitting Dounce homogenizer. This sample (~7 ml) was layered on top of a 30-ml step gradient consisting of 3 volumes of 0.6 M sorbitol and 1 volume of 0.6 M sucrose in 5 mM morpholineethanesulfonic acid (MES)-Tris (pH 6.8) and centrifuged for 1 h at 27,000 rpm in an SW-28 rotor (Beckman) (39). The pellet formed during this centrifugation step contained enriched vacuoles, whereas the purified lipid particle fraction was collected from the top of the gradient.

Characterization of subcellular fractions. (i) Marker enzymes. Invertase (10), NADPH:cytochrome *c*-reductase (30), and α-D-mannosidase (21) were assayed by established procedures.

(ii) Western blot (immunoblot) analysis. Immunological characterization of subcellular fractions was carried out after separation of proteins on sodium dodecyl sulfate (SDS)–12.5% polyacrylamide gels (14) and transfer to nitrocellulose sheets (Hybond-C; Amersham) by standard procedures (11). Proteins were detected by the enzyme-linked immunosorbent assay method with rabbit antibodies against the respective antigens and peroxidase-conjugated goat anti-rabbit secondary antibodies. Antisera against yeast plasma membrane ATPase, the 38-kDa nuclear protein, and the Kex2 protease were gifts of R. Serrano, Valencia, Spain; E. Hurt, Heidelberg, Germany; and R. Fuller, Stanford, Calif., respectively. Antibodies against porin, a protein of the outer mitochondrial membrane, were raised in rabbits as described elsewhere (7).

(iii) Lipid analyses. Ergosteryl esters and triacylglycerols as highly hydrophobic lipids are not structural components of lipid bilayer membranes. These lipids have been shown to be located almost exclusively in the so-called lipid particle fraction of yeast cells (5, 41). Thus, they are suitable markers for estimation of the relative enrichment factor of this subcellular fraction. Lipids of the isolated subcellular compartments were extracted as described by Folch et al. (9), lipid extracts were applied to silica gel plates (10 by 10 cm by 0.2 mm; Silica Gel 60; Merck, Darmstadt, Germany) with a sample applicator (Linomat IV; CAMAG, Muttenz, Switzerland), and plates were developed in an ascending manner with the solvent system light petroleum-diethyl ether-acetic acid (70:30:2, by volume). Individual neutral lipids were visualized by postchromatographic derivatization. With a chromatogram immersion device (CAMAG), plates were dipped for 8 s into the developing reagent (0.63 g of $MnCl_2 \cdot 4H_2O$, 60 ml of water, 60 ml of methanol, 4 ml of concentrated sulfuric acid), briefly dried, and heated to 120°C for 10 to 20 min. Lipids were quantified by direct densitometry at 500 nm with a Shimadzu CS 930 thin-layer chromatography scanner.

Alkaline hydrolysis of lipid extracts was done as described elsewhere (16). Individual sterols were analyzed by gas-liquid chromatography and high-performance liquid chromatography with authentic standards for identification. Gas-liquid chromatographic analysis was done with an HP 1 or HP 5 capillary column (Hewlett-Packard). The injector and detector temperatures were set at 320°C, the oven temperature was 280°C, and the nitrogen flow rate was 50 ml/min. The relative retention times of sterols were similar to those described by Nes et al. (20), Xu et al. (40), and Patterson (23). Different response factors of individual sterols were not taken into account. High-performance liquid chromatographic separation of sterols was done with an Ultrasphere ODS column (5-μm particle size; Beckman). The column was operated with methanol-water (96:4, by volume) as a solvent and a flow rate of 1.5 ml/min. Sterols were detected by measuring the A_{205}. Retention times relative to cholesterol (α_c) were identical those reported by Xu et al. (40) and Nes et al. (20).

Enzyme analyses. (i) Sterol Δ^{24}-methyltransferase. Sterol Δ^{24}-methyltransferase activity was determined after incorporation of radioactivity from *S*-adenosyl-[*methyl*-^{3}H]methionine into free sterols (19). Assays were done in a total volume of 0.5 ml containing 0.125 ml of 0.4 M Tris-Cl (pH 7.5), 0.2 to 2.0 mg of membrane protein, 0.01 ml of an ethanolic solution of unsaponifiable lipids prepared from a steryl ester fraction of *S. cerevisiae*, which contained 1 mmol of zymosterol per ml, and 0.015 ml of *S*-adenosyl-L-[*methyl*-^{3}H]methionine (100 nmol; 1.1 μCi). The reaction was linear for 3 min. After 2 min of incubation at 30°C, the reaction was terminated by addition of 5 ml of chloroform-methanol (2:1, by volume). Then, 0.75 ml of $MgCl_2$ (0.034%) and 20 μg of unlabeled carrier lipids prepared from whole yeast cells were added and the mixture was vigorously shaken. Phases were separated by centrifugation, and the organic phase was taken to dryness. Lipids were dissolved in 0.5 ml of chloroform-methanol (2:1, by volume), and aliquots were applied to thin-layer plates (10 by 10 cm by 0.2 mm; Silica Gel 60; Merck). Plates were developed with light petroleum-diethyl ether-acetic acid (70:30:2, by volume) as the solvent system. Spots corresponding to free sterols were scraped off after the plate was sprayed with water, and radioactivity was determined in Safety Cocktail (Baker) plus 5 vol% water.

(ii) Steryl ester synthase. Activity of steryl ester synthase was estimated by measuring the rate of incorporation of radioactivity from [1-^{14}C]oleoyl coenzyme A into the steryl ester fraction. An aqueous suspension of ergosterol was prepared with the nonionic detergent Triton WR 1339 (tyloxapol) (2): solutions of tyloxapol (125 mg) and ergosterol (5 mg) in acetone were mixed and taken to dryness under a stream of nitrogen at 40°C, and then 62.5 ml of 50 mM

TABLE 1. Characterization of yeast subcellular fractions

Marker	Relative enrichment (fold)[a]							
	Plasma membrane	Secretory vesicles	Vacuoles	Nucleus	Microsomes (40,000 × g)	Microsomes (100,000 × g)	Mitochondria	Lipid particles
Plasma membrane ATPase	80	2	0.3	2	2	1	0.2	~0.1[b]
Invertase	—	50	—	—	—	—	—	—
Porin	0.2	2	1	2	2	0.1	4.0	ND
38-kDa nuclear protein	—	—	—	15	—	—	—	—
NADPH:cytochrome *c* reductase	0.9	1.3	0.8	1.3	2.8	6.8	0.4	~10[b]
α-D-Mannosidase	ND	2	27	—	0.76	—	—	~20[b]
Ergosteryl esters	ND	ND	10	—	—	—	—	132
Triacylglycerols	ND	ND	12	—	—	—	—	139

[a] The specific activities of marker enzymes or the relative amounts of marker proteins of the homogenate were set at 1. ND, not detectable; —, not determined.
[b] Because of the very low protein content of lipid particles, specific activities cannot be measured accurately.

KH_2PO_4 (pH 7.0) was added to the viscous remnant. Vortexing of the mixture resulted in an almost clear micellar suspension which was stable during storage at −20°C. A standard enzyme assay (0.5-ml total volume) contained 0.25 ml of an aqueous ergosterol dispersion (see above), 0.2 to 1.5 mg of membrane protein, and 0.05 ml of [1-^{14}C]oleoyl coenzyme A (12.5 nmol; 0.1 μCi). Since oleoyl coenzyme A is a substrate for many different enzymatic reactions, the linear range of the formation of radiolabeled steryl esters is very short. Samples were taken after 30 and 60 s, and lipids were extracted and analyzed as described above. The radioactivity in steryl esters was measured.

(iii) Steryl ester hydrolase. To determine the activity of steryl ester hydrolase, some modifications were introduced into the original protocol of Taketani et al. (33). For preparation of the substrate, 0.41 mg of cholesteryloleate (10 mg/ml in chloroform-methanol; 2:1, by volume), 12.5 μCi of cholesteryl [1-^{14}C]oleate (100 μCi/ml in toluene), and 37.5 mg of Triton X-100 (100 mg/ml in acetone) were mixed and taken to complete dryness. The remnant was suspended in 6.25 ml of 0.1 M Tris-Cl (pH 7.4) and thoroughly shaken until a clear solution was obtained. Steryl ester hydrolase activity was estimated in a total volume of 0.5 ml containing 0.25 ml of the aqueous suspension of radiolabeled cholesteryl oleate (see above) and 0.2 to 2.0 mg of membrane protein. The reaction was linear with time for 60 s. Assays were stopped, lipids were extracted, and separation of neutral lipids was done as described above. Bands corresponding to free fatty acids were scraped off of the thin-layer plates, and radioactivity was determined by liquid scintillation counting.

Miscellaneous analytical procedures. Protein was quantitated by the method of Lowry et al. (17) with bovine serum albumin as the standard. The assays were done in the presence of 0.2% SDS. Proteins were routinely precipitated with trichloroacetic acid (10% final concentration) and solubilized in 0.2% SDS–0.5 M NaOH prior to determination. Since nonpolar lipids of lipid particles disturb protein measurement, these lipids were removed by extraction with diethyl ether before protein precipitation was done as described above. SDS-polyacrylamide gel electrophoresis was done by the method of Laemmli (14).

Materials. Cholesteryl [1-^{14}C]oleate (55.0 mCi/mmol), [1-^{14}C]oleoyl coenzyme A (60.0 mCi/mmol), and *S*-adenosyl-L-[*methyl*-^{3}H]methionine (10.0 Ci/mmol) were from New England Nuclear. *S*-Adenosyl-L-methionine was purchased from Boehringer Mannheim, and oleoyl coenzyme A, cholesteryloleate, cholesterol, lanosterol, ergosterol, and tyloxapol (Triton WR 1339) were from Sigma. Zymosterol was a gift of Leo W. Parks, Raleigh, N.C., and ergosta-5,7,9(11),22-tetraenol was donated by Ivan Hapala, Ivanka pri Dunaji, Slovakia. Safety Cocktail was from J. T. Baker, Deventer, The Netherlands. All of the solvents used were reagent grade.

RESULTS

Subcellular fractionation of yeast. Fractionation techniques described in Materials and Methods enabled us to isolate most yeast organellar fractions with a reasonable yield and a sufficient degree of purity (Table 1). According to organelle-specific markers, some of the membranous compartments can be obtained highly enriched over the homogenate (e.g., plasma membrane, secretory vesicles, vacuoles, and lipid particles), whereas others are less enriched and/or exhibit a higher degree of contamination. The data in Table 1 are typical for cell fractionation experiments. At least five independent preparations of all of the organelles listed were analyzed.

In contrast to those of other subcellular fractions, the enrichment and purity of lipid particles cannot be demonstrated with marker enzymes. The protein content of this fraction in very low (41), and therefore specific activities of marker enzymes could not be measured very accurately. On the other hand, ergosteryl esters and triacylglycerols are found almost exclusively in lipid particles (5, 41); these lipids can therefore serve as markers for lipid particles. As can be seen from Table 1, triacylglycerols and ergosteryl esters are equally enriched in lipid particles. Our data, however, also demonstrate that lipid particles cannot be completely removed from vacuoles and vice versa, although the method for isolation (see Materials and Methods) of these two fractions was improved. The contamination of lipid particles with microsomes also has to be taken into account. Nevertheless, the 130- to 140-fold enrichment of ergosteryl ester and triacylglycerol content in the lipid particle fraction compared with the homogenate indicates that a preparation highly enriched in this compartment was obtained.

All of the membrane fractions listed in Table 1 were also tested for contamination with Golgi membranes by using antiserum against Kex2 protease. Golgi membranes cofractionated with 40,000 × *g* microsomes. Preparations of the plasma membrane were slightly contaminated with Golgi membranes; other membrane preparations were devoid of Kex2 protein (data not shown).

Sterol composition of yeast subcellular membranes. The membrane with the highest sterol-protein and sterol-phospholipid ratios is the plasma membrane, followed by secretory vesicles (41). In the plasma membrane, ergosterol is by

TABLE 2. Sterol composition of yeast subcellular membranes

Subcellular fraction	μg of sterol/mg of organellar protein[a]						
	Ergosterol	Zymosterol	Episterol	Fecosterol	Lanosterol	Ergosta-5,7,9(11),22-tetraenol	Cholesterol
Plasma membrane	400	22	ND	ND	ND	ND	ND
Secretory vesicles	384	11	52	ND	11	8.4	ND
Microsomes (40,000 × *g*)	50	10	2.7	5.0	2.3	0.9	0.8
Microsomes (100,000 × *g*)	8.4	1.3	0.62	0.26	0.50	0.55	ND
Outer mitochondrial membrane	6.0	0.84	ND	ND	2.8	ND	2.2
Inner mitochondrial membrane	25	0.77	ND	ND	1.4	0.91	0.40
Vacuoles	49	5.9	12	2.7	4.4	2.3	ND
Lipid particles	6,690	4,390	2,870	2,180	319	121	219

[a] Data are mean values from three independent experiments with a maximum mean deviation of ±10%. ND, not detectable.

far the most prominent sterol, but a minor quantity of zymosterol was detected (Table 2). Occurrence of zymosterol in the plasma membrane is not due to contamination with other organelles, since cross-contamination is rather low (Table 1). Therefore, zymosterol must be regarded as a true component of the yeast plasma membrane. This result is in good agreement with data obtained with mammalian cells (8).

Other membranes, e.g., vacuoles or microsomes, are rather poor in sterols. These organelles contain other sterols in addition to ergosterol; among them are intermediates of the sterol biosynthetic pathway, such as lanosterol, zymosterol, fecosterol, episterol, and ergosta-5,7,9(11),22-tetraenol. The outer mitochondrial membrane of *S. cerevisiae* is almost completely devoid of ergosterol (41). Interestingly, a high percentage of unusual sterols can be detected in this membrane. Most of the mitochondrial ergosterol is located in the inner membrane of the organelle (41). A high percentage of zymosterol, ergosta-7,24(28)-dienol (episterol), and ergosta-8,24(28)-dienol (fecosterol) can be found in lipid particles. In this fraction, most of the sterols are esterified with long-chain fatty acids (data not shown); the concentration of free sterols is rather low (41).

Subcellular distribution of enzymes involved in sterol metabolism. To address the question of possible routes of sterol traffic in *S. cerevisiae*, subcellular sites of synthesis and of metabolic conversion of these lipids were localized. In higher eukaryotes, the endoplasmic reticulum is generally accepted as the site of sterol biosynthesis (1, 25, 26). Little evidence has been presented for the subcellular localization of sterol-synthesizing enzymes in *S. cerevisiae*, especially of enzymes involved in late steps of the biosynthetic pathway. To localize one of the late steps in sterol biosynthesis, we determined the subcellular distribution of sterol Δ^{24}-methyltransferase. As can be seen from Table 3, the highest specific activity of this enzyme was detected in lipid particles whereas other membranes analyzed seemed to be devoid of this enzyme. The high specific activity of this enzyme in lipid particles is due, at least in part, to the extremely low protein content of this fraction. Considering the total amount of lipid particles in yeast cells, most of the cellular capacity to catalyze this enzymatic step can be attributed to this fraction (data not shown). Enrichment of sterol Δ^{24}-methyltransferase in lipid particles confirmed earlier studies done in our laboratory (41) and by McCammon et al. (18). In contrast to previous reports (18), mitochondria were found to be devoid of this enzyme activity. This discrepancy can most likely be explained by the improved fractionation techniques used in our studies.

The subcellular localization of two other enzymes involved in sterol metabolism, steryl ester synthase and steryl ester hydrolase, is of special interest insofar as they govern the interconversion between free sterols and steryl esters. The latter components are thought to be the storage form of sterols in lipid particles, whereas free sterols are integral membrane constituents. The interconversion between free sterols and steryl esters depends on the growth state and growth conditions (16). Our data (Table 3) demonstrate that a subfraction of microsomes, namely, 40,000 × *g* microsomes, are the organelle with the highest specific activity of steryl ester synthase. Also, secretory vesicles contain substantial amounts of this enzyme, although at least part of this activity must be attributed to contamination with microsomal particles (Table 1). These results confirm earlier

TABLE 3. Subcellular distribution of sterol-metabolizing enzymes

Subcellular fraction	Sp act (nmol/min/mg)[a]		
	Sterol Δ^{24}-methyltransferase	Steryl ester synthase	Steryl ester hydrolase
Plasma membrane	0.012 ± 0.003	ND	0.45 ± 0.008
Secretory vesicles	0.14 ± 0.021	0.53 ± 0.02	0.48 ± 0.074
Microsomes (40,000 × *g*)	0.10 ± 0.001	2.54 ± 0.08	0.11 ± 0.011
Microsomes (100,000 × *g*)	0.0039 ± 0.001	0.099 ± 0.016	0.004 ± 0.001
Nucleus	0.072 ± 0.0020	0.14 ± 0.035	0.066 ± 0.016
Mitochondria	0.031 ± 0.001	0.20 ± 0.015	ND
Vacuoles	0.051 ± 0.013	ND	0.038 ± 0.002
Lipid particles	3.8 ± 0.50	ND	ND
Cytosol	—	—	ND

[a] Data were obtained from three independent experiments. ND, not detectable; —, not determined.

findings by Taketani et al. (32), who localized steryl ester synthase to microsomes. In their study, however, only nuclei, mitochondria, crude microsomes, and the cytosol were tested for the occurrence of this enzyme. Our investigations were extended to the plasma membrane, vacuoles, and lipid particles, which were shown to exhibit only minor enzyme activity.

Steryl ester hydrolase can be detected in the plasma membrane and in secretory vesicles (Table 3). Occurrence in the latter fraction was to be expected, considering the role of secretory vesicles in the well-established pathway of protein transport from the endoplasmic reticulum to the plasma membrane (4). In contrast to the results reported here, Taketani et al. (33) found the highest specific activity of steryl ester hydrolase in mitochondria. Those researchers, however, did not analyze the plasma membrane and secretory vesicles. The improved fractionation procedures used for our studies, which resulted in highly purified mitochondria, are the obvious reason for the divergent results.

DISCUSSION

The distinct subcellular distribution of free sterols and steryl esters and of enzymes involved in sterol and steryl ester metabolism points to the need for an efficient system of intracellular transport of these lipids and raises the question of possible mechanisms involved. In this respect, four essential findings described here are of special interest. (i) One of the final steps of sterol synthesis, sterol Δ^{24}-methyltransferase, is located in lipid particles. (ii) This fraction contains only traces of free sterols but large amounts of steryl esters. (iii) Esterification of sterols with long-chain fatty acids occurs in the endoplasmic reticulum, whereas hydrolysis of steryl esters takes place in secretory vesicles and in the plasma membrane. (iv) Most of the free sterols are present in the plasma membrane.

Sterol Δ^{24}-methyltransferase of lipid particles acquires its substrate, free zymosterol, from the endoplasmic reticulum, where it is synthesized. The product of the methyltransferase reaction, fecosterol, must then be translocated from lipid particles to the endoplasmic reticulum for further conversion to ergosterol. Exchange of the substrate and product could occur by collision contact between the two compartments. Alternatively, conversion of zymosterol to fecosterol could occur at contact zones between the endoplasmic reticulum and lipid particles. Close association of lipid particles and the endoplasmic reticulum has been reported before (22, 29). Steryl esters produced in the endoplasmic reticulum by steryl ester synthase have to be transferred to lipid particles, where most of the cellular steryl esters are deposited. Steryl esters represent an inert storage form of sterols that can be hydrolyzed to free sterols and fatty acids under conditions of active membrane biogenesis, e.g., when stationary-phase cells are transferred to fresh medium (34) or when sterol synthesis is inhibited. Interestingly, steryl ester hydrolase is located predominantly in the plasma membrane and in secretory vesicles. Both compartments are exceptionally rich in free ergosterol. The distinct localization of steryl ester synthase and steryl ester hydrolase at the start and end points, respectively, of cellular sterol migration suggests an alternative route of sterol transport from the site of synthesis, the endoplasmic reticulum, to the plasma membrane. This route implies steryl esters as the translocation form and lipid particles as the carrier. The fact that lipid particles are mobile, as can be seen from light microscopic inspection (40a), lends further support to this hypothesis. Studies on the structure of these lipoprotein-like particles and their biochemical function are in progress to clarify their role in the intracellular movement of sterols.

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