ATP-binding Cassette (ABC) Transporters Mediate Nonvesicular, Raft-modulated Sterol Movement from the Plasma Membrane to the Endoplasmic Reticulum*

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Little is known about the mechanisms of intracellular sterol transport or how cells maintain the high sterol concentration of the plasma membrane (PM). Here we demonstrate that two inducible ATP-binding cassette (ABC) transporters (Aus1p and Pdr11p) mediate nonvesicular movement of PM sterol to the endoplasmic reticulum (ER) in Saccharomyces cerevisiae. This transport facilitates exogenous sterol uptake, which we find requires sterol ester synthesis in the ER. Surprisingly, while expression of Aus1p and Pdr11p significantly increases sterol movement from PM to ER, it does not alter intracellular sterol distribution. Thus, ER sterol is likely rapidly returned to the PM when it is not esterified in the ER. We show that the propensity of PM sterols to be moved to the ER is largely determined by their affinity for sterol sphingolipid-enriched microdomains (rafts). Our findings suggest that raft association is a primary determinant of sterol accumulation in the PM and that Aus1p and Pdr11p facilitate sterol uptake by increasing the cycling of sterol between the PM and ER.

Maintaining the heterogeneous distribution of cholesterol among organelles is critical for a number of cellular processes including protein trafficking, signaling, and modulating membrane fluidity and permeability. Cells sustain a cholesterol gradient across the membranes of the secretory system. The lowest cholesterol concentrations are in the endoplasmic reticulum (ER) and cis-Golgi complex; higher levels are in the trans-Golgi and trans-Golgi network; and the highest amounts are in the plasma membrane (PM) (1–3). Although estimates vary, about 65–80% of the free cholesterol in cells is in the PM (1). A similar gradient, one of sphingolipids, also spans the secretory system (4). How these gradients are maintained despite continuous vesicular trafficking is not well understood, although it is likely that cholesterol and sphingolipids are actively sorted into or excluded from transport vesicles (5).

The enrichment of cholesterol and sphingolipids in the PM is also affected by the affinity of these two classes of lipid for one another because depleting cells of PM-sphingomyelin causes cholesterol in the PM to redistribute to internal compartments (6, 7). These two classes of lipid can form sterol-sphingolipid-enriched microdomains (rafts), and it has been proposed that raft association is one of the primary determinants of the intracellular distribution of sphingolipids and sterols (4). These domains, which are highly enriched in the PM but largely absent from the ER, play important roles in cell signaling and protein trafficking.

Sterols are moved between intracellular compartments by a combination of vesicular and nonvesicular mechanisms (1–3, 8). Little is known about how nonvesicular sterol transport occurs or how it contributes to maintaining the proper intracellular distribution of sterol. Here we examine sterol transport between the PM and ER in Saccharomyces cerevisiae. The intracellular distribution of the primary sterol in this yeast, ergosterol, is similar to that of cholesterol in mammalian cells (9, 10). Studies with mammalian cells have suggested that a novel transport pathway moves sterol from the ER, where it is synthesized, to the PM. ER to PM cholesterol transport does not require an intact Golgi apparatus (11–13). Treating cells with brefeldin-A, which causes the Golgi network to disassemble and inhibits protein secretion (14–16), only slightly slows the delivery of newly synthesized cholesterol to the PM. While it is possible that a novel vesicular transport pathway moves nascent cholesterol to the PM, it is more likely that ER to PM cholesterol transport is nonvesicular.

In addition to the accumulation of free sterol in the PM, many cells also have substantial amounts of fatty acyl sterol esters. Steryl esters are synthesized in the ER, but localized almost exclusively in lipid droplets.

In this study, we examine the movement of exogenous sterol from the PM to the ER in the yeast S. cerevisiae. Since this yeast does not take up sterol when grown aerobically, we use strains with an altered allele of a transcription factor (upc2-1) that allows cells to take up sterol during aerobic growth. Microarray analysis has revealed that upc2-1 increases the expression of a large number of genes including AUS1 and PDR11, which encode ATP-binding cassette (ABC) transporters (17). Deleting these two genes substantially reduced the ability of a upc2-1 strain to take up exogenous sterol. Here we examine the mechanism of these transporters and how they affect intracellular sterol distribution. We find they facilitate sterol cycling between the PM and ER. This cycling likely promotes exogenous sterol uptake, since we also find that sterol ester synthesis in the ER is required for efficient exogenous sterol uptake. Thus, Aus1p and Pdr11p increase the availability of PM sterol for esterification in the ER, which in turn contributes to the net uptake of exogenous sterol by cells. In addition, we also find that the propensity of a sterol to be moved between the PM and ER is largely determined by its raft affinity, suggesting that raft association is a primary determinant of sterol accumulation in the PM.
Sterol uptake was monitored and quantitated as described above. The membrane potential of the cells was measured using an Oxford Instrument Premier 6.0 equipment. The signals were recorded using a computerized data acquisition system (Oxford Instruments). The membrane potential was calculated using the Nernst equation, and the results were expressed as the difference between the membrane potential in the presence and absence of 20 μM valinomycin.

Immunoblot analysis of the protein content was performed as described previously (25). Whole-cell extracts were prepared as described in MATERIALS AND METHODS, and equal amounts of protein were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were probed with primary antibodies raised against Pep12p (25), a protein involved in the uptake of sterols at the plasma membrane (27), and Vps10p, a protein involved in the transport of sterols into the ER (26). Horseradish peroxidase-conjugated secondary antibodies were used to detect the primary antibodies, and the signals were visualized using an enhanced chemiluminescence detection system (Amersham Biosciences). The intensity of the bands was quantitated using a Bio-Rad Gel Doc 2000 system.

Results

The experiments were performed as described previously (25). Briefly, cells were grown to mid-log phase in YPD medium containing 0.5% Tween 80, and then treated with 20 μM valinomycin. The cells were harvested by centrifugation, and the membrane potential was measured as described above. The results were expressed as the difference between the membrane potential in the presence and absence of 20 μM valinomycin.

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Nonvesicular Transport of Plasma Membrane Sterols to the ER

RESULTS

Exogenous Sterols Are Transported from the PM to the ER at Different Rates—To study how sterol is moved between the PM and ER, we exploited the ER localization of the enzymes that convert free sterol to fatty acyl sterol esters (29). The esterification of exogenous sterol indicates that it has been transported to the ER and esterified was not significantly affected by the amount of sterol taken up by the cells. We were not able to saturate [14C]cholesterol uptake because of the limited solubility of cholesterol in the medium.

In contrast to the rapid esterification of exogenous [14C]cholesterol, only a small fraction of the sterol taken up by the cells became esterified when cells were grown [14C]ergosterol. Over a range of concentrations of exogenous [14C]ergosterol, only 14% of the sterol taken up became esterified 30 min after addition to the medium (Fig. 1, G–I). We also determined the rate of esterification of exogenous [3H]sitosterol and 7-dehydrocholesterol (7-DHC) and found that they were esterified at rates intermediate between those of [14C]cholesterol and [14C]ergosterol (Fig. 1, E and F).

Yeast is able to grow with all of the four sterols used in this study (ergosterol, cholesterol, sitosterol, and 7-DHC). A strain that cannot synthesize sterol, and therefore requires exogenous sterol supplementation, grows at about the same rate when any of these four sterols is added to the medium (not shown).

Exogenous sterol that has been taken up but has not become esterified remains primarily in the PM-enriched fractions of density gradients. We determined the intracellular distribution of exogenous ergosterol and 7-DHC, two sterols that are slowly esterified, by fractionating total cellular membranes on Renografin-76 density gradients. Almost all of the exogenous ergosterol and 7-DHC that had not been esterified remained in the PM-enriched fractions (Fig. 2, A and C). Thus, after uptake, sterol that has not become esterified remains largely in the PM.

The different esterification rates of exogenous sterols likely reflect disparities in their transport rate to the ER, and not differences in the affinity of yeast sterol ester synthases (Are1p and Are2p) for the sterols. We found that microsomes derived from the upc2-1 strain used in this study (WPY361) were able to esterify various sterols at similar rates (Table II). In agreement with previous findings (26), there was a 2-fold difference in the rate of cholesteryl and ergosteryl ester synthesis by microsomes. Since there is about a 6-fold difference between the rates at which exogenous cholesterol and ergosterol are esterified, differences in the rates of exogenous sterol esterification are probably primarily the result of disparities in their transport rates to the ER. Thus, after uptake, exogenous free sterols remain largely in the PM and are likely transported to the ER at different rates.

Nonvesicular Sterol Transport between the PM and ER—To determine if PM to ER sterol transport requires vesicular transport, we looked at the rate of sterol transport in a number of mutants with conditional defects in proteins required for vesicular trafficking. Sec18p, the yeast homolog of N-ethylmaleimide-sensitive factor, is required for most if not all vesicular transport (30, 31). The transport rate of exogenous cholesterol to the ER was not substantially affected in cells with a temperature-sensitive defect in this protein after the cells were grown for 60 min at nonpermissive temperature (Fig. 3), a treatment which results in a severe defect in vesicular transport (31). Vesicular trafficking to and from the ER also requires the coat-forming protein complexes COPI and COPII (32). However, sterol transport from the PM to the ER was not affected in mutants with conditional defects in proteins required for the formation of these complexes after 1 h at nonpermissive temperature (Fig. 3, bottom two panels). Similar results were obtained with a number of other mutants with conditional defects in proteins needed for vesicular trafficking at other steps of the secretory and endocytic pathways including: Sec4p, Sec6p, Sec8p, Sec15p, and Act1p (not shown). Thus, PM sterol can be transported to the ER by a pathway that does not require any one of a number of proteins required for vesicular trafficking to the ER or other cell compartments. Although it is possible that such transport occurs in some as yet undiscovered vesicles that use a novel transport machinery, it is more likely that the transport is nonvesicular. This transport could be mediated by soluble carrier proteins, membrane proteins at points of ER-PM membrane apposition, or diffusion through the aqueous phase.

Aus1p and Pdr11p Are Localized to the PM—The ABC transporters Aus1p and Pdr11p have been implicated in sterol uptake (17). These transporters are expressed at very low levels
in cells grown aerobically. However, their expression is induced in cells with an altered allele of a transcription factor (upc2-1).

Cells with the upc2-1 allele that also have deletions of AUS1 and PDR11 (upc2-1/H9004 aus1/H9004 pdr11) take up significantly less exogenous cholesterol than upc2-1 cells (17).

In order to learn more about the potential function of these transporters in sterol uptake and trafficking, we constructed plasmids that encode C-terminal fusions of these proteins to GFP. These fusion proteins are functional since they allow a upc2-1/H9004 aus1/H9004 pdr11 strain to take up exogenous sterol (not shown). We found that both Aus1p and Pdr11p localize to the PM (Fig. 4). Interestingly, a significant fraction of Aus1-GFP is also in unidentified internal compartments.

**Fig. 2.** The intracellular distribution of free sterols is not altered in upc2-1 cells. Total cellular membranes were fractionated on RenoCal-76 density gradients and the free (i.e. non-esterified) sterol concentration in each fraction determined as described under “Experimental Procedures.” A, distribution of exogenous free sterols in WPy361 (upc2-1) 30 min after addition to the medium at 50 μM. B, steady-state distribution of sterols. Strain WPy361 was grown for 14–20 h in medium supplemented with either 1 mM [14C]acetate (to measure endogenous sterols) or 20 μg/ml [14C]cholesterol or [14C]ergosterol (to assess exogenous sterol distribution). C, fractions from gradients were immunoblotted with anti-Pma1p (PM), anti-Sec61p (ER), anti-Pep12p (endosomes), and anti-Vps10p (late-Golgi complex) antibodies.

**Fig. 3.** PM to ER sterol transport is nonvesicular. The indicated strains were grown at 23 °C (permissive temperature) and then shifted to 37 °C (non-permissive temperature) for 60 min. [14C]Cholesterol (2 μM) was added to the medium, and the amount taken up and esterified was determined as described under “Experimental Procedures.” The amounts of free, esterified, and total sterol taken up are indicated as in Fig. 1.

**Fig. 4.** Aus1p and Pdr11p are in the PM. A upc2-1 strain (WPy361) was grown with plasmids encoding C-terminal fusions of GFP to either Aus1p or Pdr11p, each under their own promoter. Fluorescence (GFP) and Nomarski images of the cells are shown. Bars are 1 μm.

**Rapid PM to ER Sterol Transport Requires Aus1p or Pdr11p**—We wanted to investigate the role of Aus1p and Pdr11p in sterol uptake and trafficking. Since the upc2-1 allele, that allows sterol uptake during aerobic growth, increases the expression of a large number of genes including AUS1 and PDR11 (17), we wanted to rule out that knock-out AUS1 and PDR11 in upc2-1 cells indirectly affects sterol uptake by turning down the expression of other genes induced by upc2-1. A plasmid (pAUS1-MEL1) was constructed in which the promoter of AUS1 drives the expression of the reporter gene MEL1, which encodes β-galactosidase. UPC2 cells (i.e. wild-type) containing this plasmid express very little β-galactosidase, whereas the upc2-1 strain (WPY361) increases the expression by more than 350 times (Fig. 5A, left panel). When we deleted AUS1 and PDR11 in WPy361, β-galactosidase expression from pAUS1-MEL1 decreased only slightly. Thus, upc2-1 Δaus1 Δpdr11 cells probably do not fail to take up exogenous sterols.
were taken at the indicated time, and the amount of esterified of the indicated strains, and the amount taken up and esterified was measured (right panel). The activities shown are the average of two determinations. B, 2 μm [14C]cholesterol was added to the medium of the indicated strains, and the amount taken up and esterified was determined as described above. C, growing cultures of upc2-1 (squares) and upc2-1 Δpdr11 Δaus1 (triangles) cells were depleted of ATP with azide (30 mM) and 2-deoxyglucose (30 mM) for 5 min, and 10 μM [14C]cholesterol was then added to the medium. After 15 min, the cells were washed in medium containing 0.5% Tween 80 and resuspended in fresh medium without supplementation (open symbols) or with 50 μM unlabeled cholesterol (closed symbols). Samples were taken at the indicated time, and the amount of esterified [14C]cholesterol determined.

sterol because they down regulate other genes that would otherwise be induced by the upc2-1 allele. We determined the rate of [14C]cholesterol esterification in strains missing either or both of these transporters, in order to assess the role of Aus1p and Pdr11p in intracellular sterol trafficking. As expected, cells missing both transporters (upc2-1 aus1 Δpdr11) took up much less [14C]cholesterol from the medium than an isogenic upc2-1 strain (Fig. 5B). Surprisingly, only a small fraction of the sterol taken up by the upc2-1 Δaus1 Δpdr11 strain was esterified. This difference could not be explained by the differences in the amount of sterol taken up by the upc2-1 and the upc2-1 Δaus1 Δpdr11 strains. As mentioned, the percent of the total cholesterol taken up that becomes esterified is very similar over a range of exogenous sterol concentrations (Fig. 1, A–D). Additionally, when the upc2-1 strain is presented with a lower amount of cholesterol (0.2 μM, Fig. 1A), it takes up an amount of cholesterol that is comparable to the amount taken up by the upc2-1 Δaus1 Δpdr11 strain given a higher concentration (2 μM, Fig. 5B, lower right panel) and yet only a small fraction of the exogenous cholesterol becomes esterified in the upc2-1 Δaus1 Δpdr11 strain. To rule out that this difference was caused by differences in the ability of upc2-1 and upc2-1 Δaus1 Δpdr11 cells to esterify sterol, we determined the sterol ester synthase activity of microsomes from both strains. Knocking-out AUS1 and PDR11 in the upc2-1 strain does not alter its ability to esterify cholesterol (Fig. 5A, right panel).

These findings suggested that Aus1p and Pdr11p facilitate the transport of exogenous [14C]cholesterol to the ER and not simply the uptake of exogenous sterol into the PM. The functions of the two transporters seem to overlap since cells missing only one of the transporters had only modest defects in the amount of [14C]cholesterol taken up and in the extent of esterification (Fig. 5B).

A possible function of the transporters could be to facilitate the movement of exogenous sterol from the periplasm, across the PM, and into the cytosol or the ER. Alternatively, the transporters might facilitate the movement of sterol already in the PM to internal compartments. To distinguish between these possibilities, we inhibited the transporters, allowed [14C]cholesterol to diffuse into the PM, washed the cells, and then determined the esterification rate of the [14C]cholesterol by upc2-1 and upc2-1 Δaus1 Δpdr11 cells. After depletion of ATP by treatment with 2-deoxyglucose and NaN3 for 5 min, radiolabeled cholesterol was added and allowed to diffuse into the PM for 15 min. The cells were washed with medium containing the detergent used to deliver the [14C]cholesterol to the cells and resuspended in fresh medium. After this treatment, the upc2-1 and upc2-1 Δaus1 Δpdr11 cells took up the same amount of [14C]cholesterol, 15 ± 3.1 and 14 ± 2.5 pmol/OD600 respectively. Almost none of the [14C]-cholesterol taken up while ATP was depleted became esterified (Fig. 5C, time 0). After the cells recovered from ATP-depletion, a fraction of the [14C]cholesterol in the upc2-1 strain became esterified, while almost none was esterified by the upc2-1 Δaus1 Δpdr11 strain (Fig. 5C). Thus, Aus1p and Pdr11p likely facilitate the movement of PM sterol to the ER.

We wanted to rule out that the esterification of [14C]cholesterol by the upc2-1 strain in this experiment was the result of [14C]cholesterol efflux into the medium and subsequent re-uptake. We performed the same experiment but added 50 μM unlabeled cholesterol to the medium after washing the cells to dilute out any [14C]cholesterol that effluxed into the medium. Since addition of unlabeled cholesterol actually increases the rate of esterification ([14C]cholesterol (Fig. 5C), the esterification of [14C]cholesterol in these experiments is probably not the result of efflux and reuptake.

Sterol Uptake Requires Sterol Ester Synthase—Our findings suggest that Aus1p and Pdr11p increase the rate at which PM sterol reaches the ER, where it becomes available for esterification. We wondered if sterol esterification in the ER was necessary for sterol accumulation by cells. Since esterified sterol is stored in lipid particles, esterification could act as a sink that causes the net uptake of sterol into cells. A strain that has the upc2-1 allele, but which is also missing the genes that encode the two steryl ester synthases in yeast (upc2-1 Δare1 Δare2), does not take up much more exogenous sterol than a strain lacking the upc2-1 allele (Fig. 6A). We wanted to rule out that this decrease in uptake occurs because genes turned on in upc2-1 cells are turned off again in upc2-1 Δare1 Δare2 cells. To determine if genes turned on in upc2-1 cells are also on in upc2-1 Δare1 Δare2 cells, we introduced pAUS1-MEL1 into these strains. The expression of this reporter was substantially lower in upc2-1 Δare1 Δare2 cells compared with an isogenic upc2-1 strain (Fig. 6B). However, the level of α-galactosidase expressed by the upc2-1 Δare1 Δare2 strain is comparable to other upc2-1 strains that can still take up exogenous sterol. It has previously been shown that some strains containing the upc2-1 allele can take up substantially more sterol than others, probably because of one or more as yet uncharacterized mutations (17). Thus, the upc2-1 Δare1 Δare2 strain expresses genes
Sterol uptake requires steryl ester synthase. A, indicated strains were grown with 50 μM [14C]cholesterol in medium containing 0.5% Tween 80 for 2 h. The cells were washed with ice-cold 10 mM NaN3 containing 0.5% Tween 80 and the total amount of cholesterol taken up was determined by scintillation counting. B, pAUS1-MEL1 plasmid as introduced into cells and the α-galactosidase activity determined as described under “Experimental Procedures.”

up-regulated by upc2-1 at levels similar to some other upc2-1 strains, but cannot take up exogenous sterol as well as them. Since these findings suggest that steryl ester synthase activity is required for sterol uptake, it is likely that Aus1p and Pdr11p, by themselves, do not cause sterol accumulation in cells. Instead, their role would be increasing the rate at which PM sterol becomes available for esterification in the ER.

PM to ER Sterol Transport Rate Correlates with Raft Affinity—Our findings suggest that various exogenous sterols are moved between the PM and ER at different rates. One explanation for these differences may be that Aus1p and Pdr11p, or other proteins required for nonvesicular sterol transport, have different affinities for sterols. It is equally possible that the intracellular transport rates of sterols are affected by their ability to become raft associated. In vitro studies suggest that sterols have different propensities to form rafts (33). In addition, it has long been known that depleting the PM of mammalian cells of sphingomyelin causes cholesterol in the PM to redistribute to internal compartments (6, 7). Therefore, the distribution and transport of sterols may be affected by their ability to associate with sphingolipids and form rafts in the PM. When exogenous sterol enters the PM, it likely competes with endogenous sterol for raft association. If raft-associated PM sterol is not available for nonvesicular PM to ER transport, a sterol with high raft affinity would be moved more slowly from PM to ER than one with low affinity. Alternatively, the relative affinity of a sterol for rafts might affect the rate at which it is moved back to the PM after it has been removed. Differences in the rates of esterification of exogenous sterols may therefore partially reflect their relative ability to compete with endogenous ergosterol for raft association.

We wanted to estimate the ability of various exogenous sterols to compete with endogenous sterol to become raft-associated in the PM in vitro. Lipids and proteins in rafts can be detected by their insolubility in cold 1% Triton X-100 (34). While the amount of lipids in the insoluble fraction may not be exactly the same as that in native membranes, their degree of insolubility correlates with their relative proportion in rafts (35). To estimate what fraction of PM sterols are in rafts, we grew yeast with exogenous sterols, isolated total cellular membranes, obtained the PM-enriched fractions from RenoCal-76 density gradients, and determined the percentage of sterol in these that is resistant to cold Triton X-100 extraction from the membranes. Consistent with previously published values (36), we found that about 65% of endogenous ergosterol in the PM-enriched fractions is detergent resistant (Fig. 7A, column 1). Not surprisingly, a similar amount of the exogenous ergosterol in the PM (about 58%) was also detergent resistant (Fig. 7, column 2). In contrast, only a small fraction of exogenous cholesterol (16%) in the PM was in rafts (column 3). The amount of exogenous sitosterol and 7-DHC in PM rafts (28 and 49%, respectively) was between the values for exogenous cholesterol and ergosterol (columns 4 and 5). There is, then, a good correlation between the tendency of a sterol to become raft-associated in the PM (i.e. resistant to Triton X-100 extraction) and its rate of esterification during uptake (Fig. 7B). Thus, raft association may affect the rate of sterol transport between the PM and ER. A sterol such as cholesterol, which has lower raft affinity than endogenous ergosterol, will tend to remain non-raft-associated in the PM, leading to a greater fraction available for transport to the ER and esterification there.

Depleting Cells of Sphingolipids Alters the Rate of Sterol Transport between the PM and ER—Our findings suggest that the difference in the rates at which exogenous ergosterol and cholesterol are esterified is primarily determined by their relative ability to compete for raft association. Depleting cells of sphingolipids and, in turn, rafts should therefore cause the difference in esterification rate of exogenous ergosterol and cholesterol to narrow. We depleted cells of sphingolipid by treating them for 2 h with myriocin, which inhibits serine palmitoyltransferase (37), the first step in ceramide and sphingolipid biosynthesis. After myriocin treatment, only a small fraction of the endogenous ergosterol in the PM was resistant to extraction with TX100 (Fig. 7, column 6). Raft depletion did not occur in cells given both myriocin and the ceramide precursor dihydrosphingosine (not shown). In untreated cells there is a 5.9-fold difference in the rate at which exogenous ergosterol and cholesterol are esterified. As predicted, this difference narrows to 3.0-fold after depletion of sphingolipids with myriocin (Fig. 8, A and B). Thus, depleting cells of sphingolipids decreases the difference in the rates at which exogenous cholesterol and ergosterol are transported to the ER and esterified. The relative affinity of exogenous sterol for PM rafts likely affects either the fraction of sterol that is available for nonvesicular transport to the ER or the rate at which sterol in the ER moves back to the PM.

The decrease in the esterification rates of exogenous chole-
terol and ergosterol after myriocin treatment may occur for two reasons. First, raft depletion likely increases the amount of endogenous sterol available for esterification. This endogenous sterol may complete with exogenous sterol for esterification and reduce the esterification rate of exogenous sterol. Second, it is possible that myriocin treatment decreases the activity of the yeast sterol ester synthases.

**Alter the Relative Affinity of Cholesterol for Rafts Changes Its PM to ER Transport Rate**—Exogenous cholesterol probably remains largely non-raft-associated in the PM because it has a lower affinity for rafts than ergosterol, the primary endogenous PM sterol. Exogenous cholesterol likely cannot compete with endogenous ergosterol for raft binding. The situation should be different, however, in cells that contain cholesterol rather than ergosterol as their primary sterol. In this case, exogenous cholesterol will not be outcompeted for raft association by PM sterol and should be transported to the ER much more slowly than in cells with ergosterol as the primary PM sterol. To generate yeast with cholesterol, rather than ergosterol, as the primary PM sterol, we used a yeast strain (CP3) that cannot synthesize sterol because it has a deletion of *ERG1* and requires exogenous sterol for growth. This strain grows at about the same rate whether it is supplemented with ergosterol or cholesterol (not shown). Grown with cholesterol, the rate of cholesterol transport from the PM to the ER was much lower in CP3 than when it was grown with ergosterol (Fig. 8C). As predicted, the rate of cholesterol transport to the ER in CP3 grown with cholesterol was very similar to the rate of ergosterol transport in a strain that makes its own ergosterol (Fig. 1, G–I). Taken together, these results suggest that the ability of exogenous sterol to compete for raft association dramatically affects the rate at which it is transported between the PM and ER.

**Aus1p and Pdr11p Do Not Alter the Distribution of Endogenous Sterol**—Because Aus1p and Pdr11p facilitate the movement of sterol from the PM to the ER, expression of these transporters may alter the intracellular distribution of free (i.e., non-esterified) sterols. In yeast, and higher eukaryotes, most of the free sterol in the cell is in the PM. To look at the relative distribution of free sterol between the PM and internal compartments, cells labeled with [14C]acetate were fractionated on RenoCal-76 density gradients, and the amount of free sterol in each fraction determined. In a UPC2 (wild-type) strain, in which expression of Aus1p and Pdr11p is not induced, most of the free sterol is in PM-enriched fractions (Fig. 2, B and C and Ref. 19). Despite the ability of Aus1p and Pdr11p to facilitate PM to ER sterol transport, intracellular distribution of endogenous sterol was not altered in cells that express the transporters (upc2-1). One explanation could be that the transporters act only on exogenous sterol in the PM and therefore do not affect the distribution of endogenous sterol. However, it seems more likely that they do facilitate the movement of endogenous PM sterol as well. After sterol is dislocated from the PM by Aus1p or Pdr11p it seems probable that it rapidly moves back to the PM if it is not esterified in the ER.

We also looked at the steady-state intracellular distribution of exogenous free sterols in cells grown for 8–10 generations in the presence of either [14C]cholesterol or [14C]ergosterol. It should be noted that yeast continues to make endogenous sterol even when it takes up exogenous sterol from the medium. Not surprisingly, we found that the intracellular distribution of exogenous free ergosterol is very similar to that of endogenous sterol, which is primarily ergosterol (Fig. 2B). In contrast, a greater fraction of exogenous free cholesterol was enriched in internal membranes. Thus, cholesterol, which is outcompeted for raft association by endogenous ergosterol, is less enriched in the PM than endogenous sterol. It is likely that the relative affinity of a sterol for rafts affects its distribution between the PM and the ER.

**DISCUSSION**

Here we demonstrate that two ABC transporters facilitate nonvesicular sterol movement from the PM to the ER. While ABC transporters have previously been shown to be required for sterol efflux from cells (38–41), this is the first demonstration that they can also facilitate intracellular sterol transport. Whether Aus1p and Pdr11p directly or indirectly cause sterol to move to the ER remains to be determined but a number of mechanisms may apply. For example, the transporters may move sterol directly out of the PM either into the aqueous phase or to soluble sterol-binding proteins. It is also possible that they catalyze the movement of sterols from the outer to the inner leaflet of the PM bilayer, although, this seems unlikely since most evidence suggests that sterols rapidly move spontaneously between bilayer leaflets (42). Aus1p and Pdr11p could also indirectly facilitate sterol movement from the PM to the ER. For example, they might catalyze the movement of another lipid and indirectly cause sterol to move to the ER. Such a mechanism has been suggested for ABCA1, an ABC transporter that is required for cholesterol efflux to in mammalian cells (43). Another possibility is that Aus1p and Pdr11p facilitate contacts between the PM and ER and so allow sterols to more rapidly diffuse between the two compartments. It is not
yet know if these transporters facilitate nonvesicular sterol transport to other organelles as well.

It is likely that one of the primary functions of Aus1p and Pdr11p is to facilitate the movement of PM sterol to the ER where it can become esterified, since we find that sterol esterification is necessary for efficient sterol uptake. Without Aus1p and Pdr11p, the rate of PM sterol transport to the ER does not appear to be sufficiently rapid to facilitate sterol uptake. Thus, in the absence of these transporters, exogenous PM sterol is transported to the ER only very slowly. In mammalian cells, the estimated half-time of sterol cycling between the PM and ER is forty minutes (44). Our results suggest that, in the absence of Aus1p and Pdr11p, this rate may be slower in yeast.

We do not yet know if the path of sterol from PM to ER without the transporters is exclusively vesicular or not. Since a nonvesicular transport pathway moves sterol from the PM to the ER in mammalian cells (11–13), it is likely that it can also move PM sterol to the ER. Thus, it appears that Aus1p and Pdr11p are not required for nonvesicular PM to ER sterol transport, but rather increase the rate at which this transport occurs. The identification of other proteins in this nonvesicular transport pathway is underway.

Whether Aus1p, Pdr11p, or other transporters, also catalyze the movement of exogenous sterol from the periplasm into the PM remains to be determined. However, it may be that yeast does not actively transport exogenous sterol into the PM to avoid sterol toxicity. Instead, sterols may simply passively diffuse from the medium into the PM.

Why would cells need ATP-requiring transporters to facilitate the movement of sterol from the PM, where free sterol concentration is high, to the ER, where it is relatively low? While it is not known what drives the net accumulation of sterol in the PM, it is likely that Aus1p and Pdr11p are needed to move sterol against this force (Fig. 9). It has been proposed that sterols and sphingolipids accumulate in the PM because they, or more likely rafts, are enriched in anterograde vesicles in the secretory system and depleted from retrograde vesicles (4). After Aus1p or Pdr11p has moved a PM sterol to the ER, its raft affinity is expected to cause it to be rapidly returned to the PM if it is not esterified in the ER. Because the raft affinity of free sterols likely causes them to be rapidly concentrated in the PM, Aus1p and Pdr11p effectively increase the rate at which PM sterols cycle back through the ER.

Consistent with this conclusion, our findings suggest that the relative raft affinity of sterols affects the rates at which they are moved to the ER and esterified. This likely occurs for two reasons. First, the relative affinity of a sterol in the PM for rafts may determine the fraction of sterol that is available for nonvesicular transport to the ER. For example, if only nonraft-associated sterol is available for nonvesicular transport to the ER, then the pool of PM sterol available for movement to the ER will be affected by the relative raft affinity of a sterol. Once sterol has been moved to the ER, the rate at which it is moved back to the PM may also be determined by its relative affinity for rafts. A sterol with low raft affinity will tend to remain in the ER longer and thus become esterified more rapidly than a sterol with high raft affinity.

The raft affinity of a sterol not only affects its transport rate between the PM and ER, but its intracellular distribution as well. Treating mammalian cells with sphingomyelinses causes cholesterol to redistribute to the internal compartments (6, 7). Consistent with these findings, we show that cholesterol, which remains largely nonraft-associated in the yeast PM, is more enriched in internal compartments than is endogenous sterol (Fig. 2B). Thus, raft association is likely an important determinant of sterol distribution within the cell.

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