Sterol Carrier Protein-2 Expression Alters Plasma Membrane Lipid Distribution and Cholesterol Dynamics†

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ABSTRACT: Although sterol carrier protein-2 (SCP-2) binds, transfers, and/or enhances the metabolism of many membrane lipid species (fatty acids, cholesterol, phospholipids), it is not known if SCP-2 expression actually alters the membrane distribution of lipids in living cells or tissues. As shown herein for the first time, expression of SCP-2 in transfected L-cell fibroblasts reduced the plasma membrane levels of lipid species known to traffic through the HDL-receptor-mediated efflux pathway: cholesterol, cholesteryl esters, and phospholipids. While the ratio of cholesterol/phospholipid in plasma membranes of intact cells was not changed by SCP-2 expression, phosphatidylinositol, a molecule important to intracellular signaling and vesicular trafficking, and anionic phospholipids were selectively retained. Only modest alterations in plasma membrane phospholipid percent fatty acid composition but no overall change in the proportion of saturated, unsaturated, monounsaturated, or polyunsaturated fatty acids were observed. The reduced plasma membrane content of cholesterol was not due to SCP-2 inhibition of sterol transfer from the lysosomes to the plasma membranes. SCP-2 dramatically enhanced sterol transfer from isolated lysosomal membranes to plasma membranes by eliciting detectable sterol transfer within 30 s, decreasing the $t_{1/2}$ for sterol transfer 364-fold from >4 days to 7−15 min, and inducing formation of rapidly transferable sterol domains. In summary, data obtained with intact transfected cells and in vitro sterol transfer assays showed that SCP-2 expression (i) selectively modulated plasma membrane lipid composition and (ii) decreased the plasma membrane content cholesterol, an effect potentially due to more rapid SCP-2-mediated cholesterol transfer from versus to the plasma membrane.

Exogenous lipids enter and leave mammalian cells via plasma membrane receptors for serum lipoproteins such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Two very different pathways are essential to maintaining cellular lipid homeostasis. The LDL-receptor endocytic pathway is primarily responsible for the endocytic uptake of exogenous unesterified cholesterol, as well as fatty acid esterified to cholesterol, phospholipid, and triglycerides (reviewed in refs *1*−*4*). For some cell types the HDL-receptor pathway represents an alternate, but nonendocytic, mechanism for molecular uptake of cholesteryl esters (*5*−*7*), cholesterol (*8*), and phospholipids (*7*). In contrast to the LDL-receptor pathway, the HDL-receptor pathway can also operate in the reverse direction to mediate "reverse cholesterol transport", i.e., cellular cholesterol efflux (reviewed in refs *2*, *9*, and *10*).

While many significant advances have been made in our understanding of the intracellular factors regulating the LDL-receptor endocytic pathway, much less is known regarding intracellular factors (lipid binding proteins) involved in HDL-receptor-mediated, nonendocytic, lipid uptake and efflux through the plasma membrane. A potential candidate protein is sterol carrier protein-2 (SCP-2),[1] a protein whose ligand binding specificity and intracellular distribution place it in a position to dramatically alter cellular lipid dynamics. SCP-2 exhibits broad ligand specificity for fatty acids (*11*−*14*), fatty acyl CoAs (*15*, *16*), and cholesterol (*13*, *17*−*20*). SCP-2 is ubiquitously distributed among all tissues examined with especially high levels in liver and steroidogenic cells (reviewed in refs *19* and *21*−*24*). Although SCP-2 is localized in highest concentration in peroxisomes, significant quantities of SCP-2 are localized outside the peroxisomes of essentially all tissues examined by immunogold electron microscopy: liver, steroidogenic cells, and intestine (*25*−*32*) and transfected hepatoma cells expressing SCP-2 (*33*). Quantitative analysis of the immunogold electron microscopic observations, taken together with the fact that <1% of cell volume is occupied by peroxisomes, indicated that nearly half of SCP-2 is extraperoxisomal and localized in the cytoplasm, mitochondria, and endoplasmic reticulum of these tissues and cells (reviewed in ref *19*). Using a totally different technology, immunofluorescence confocal imaging, it was confirmed that at least 50% of SCP-2 is extraperoxisomal in transfected cells (L-cells, hepatoma) expressing SCP-2 (*34*−*36*). These findings were also substantiated by

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[1] Abbreviations: SCP-2, sterol carrier protein-2; DHE, dehydroergosterol; PBS, phosphate-buffered saline; BHT, butylated hydroxytoluene; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine; CerPCho, sphingomyelin.

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earlier subcellular fractionation studies (*29*, *37*). Thus, SCP-2 is distributed, at least in part, within the cell such that it may influence uptake, diffusion, and metabolism of ligands which it binds. Indeed, SCP-2 expression stimulates cholesterol uptake (LDL-receptor mediated) 1.3-fold and intracellular transfer of plasma membrane cholesterol to the endoplasmic reticulum 11-fold for esterification by acyl CoA cholesterol acyltransferase (ACAT) (*38*, *39*). Concomitantly, SCP-2 inhibits HDL-receptor-mediated cholesterol efflux from lipid droplets to plasma membrane caveolae (*40*). Finally, SCP-2 expression enhances the uptake (*19*, *41*) and intracellular diffusion (*19*, *41*) of fatty acids in intact cells and incorporation of fatty acids into phospholipids in vitro (*35*, *42*). Nevertheless, it is not known whether expression of SCP-2 can alter the plasma membrane equilibrium distribution of lipids.

Earlier data with another lipid binding protein (liver fatty acid binding protein, L-FABP) suggest that SCP-2 may indeed alter plasma membrane lipid distribution. Like SCP-2, L-FABP also binds fatty acids (reviewed in ref *43*), fatty acyl CoAs (*44*), and cholesterol (*44*−*46*). L-FABP stimulates the intracellular transfer of plasma membrane cholesterol to the endoplasmic reticulum 1.2-fold for esterification by ACAT (*47*). L-FABP expression also stimulates the uptake (reviewed in refs *43* and *48*) and intracellular diffusion (*48*) of fatty acids in intact cells as well as the incorporation of fatty acyl CoAs into membrane phospholipids in vitro (*49*−*51*). L-FABP expression reduces the plasma membrane cholesterol content and cholesterol/phospholipid ratio by 1.9- and 1.6-fold, respectively, while eliciting only minor changes in phospholipid composition (*47*, *52*). The L-FABP expression-induced decrease in the plasma membrane cholesterol/phospholipid ratio results in more fluid L-cell plasma membranes and decreases plasma membrane Na^+K^+-ATPase activity. Although L-cell growth and doubling time are not inhibited by L-FABP expression (*47*), L-FABP expression in embryonic stem cells stimulates cell differentiation (*53*). Interestingly, when the SCP-x/SCP-2 gene is knocked out in transgenic mice, compensatory upregulation of L-FABP was observed (*16*, *54*). The many similarities in L-FABP and SCP-2 ligand binding profiles, effects on ligand diffusion, stimulation of ligand incorporation into phospholipids, and compensatory regulation in mice suggest that SCP-2 expression may also alter the lipid distribution to the plasma membrane.

In summary, intracellular proteins such as SCP-2 interact with and transfer many lipidic molecules including fatty acids, fatty acyl CoAs, cholesterol, and phospholipids in vitro. Results in intact cells (*33*, *40*, *55*, *56*) and animals (reviewed in refs *16*, *54*, and *57*−*63*) indicate that SCP-2 is physiologically important in fatty acid, phospholipid, and sterol trafficking and metabolism. Many of the lipids (cholesterol, phospholipids, cholesteryl esters, fatty acids) that pass through the plasma membrane by molecular transfer via the HDL-receptor pathway are also ligands for SCP-2. The present investigation was to determine (i) if SCP-2 expression in transfected cells could alter the lipid distribution to the plasma membrane and (ii) if altered distribution of one such lipid, cholesterol, was due to inhibition of transfer by SCP-2. The data showed that SCP-2 expression redistributed cholesterol away from the plasma membrane, an effect that was not due to SCP-2-mediated inhibition of cholesterol transfer from lysosomal membranes to plasma membranes. SCP-2 expression differentially redistributed phospholipid away from the plasma membrane but selectively retained phosphatidylinositol. SCP-2 overexpression did not alter the percent composition of the fatty acids esterified to the plasma membrane phospholipids. These data show for the first time that SCP-2 expression can alter the lipid distribution to any cellular membrane.

MATERIALS AND METHODS

Materials. Dehydroergosterol (DHE) was synthesized as described (*64*) or purchased from Sigma Chemical Co. (St. Louis, MO) and used without additional purification. Sucrose and Percoll were purchased from Sigma Chemical Co. (St. Louis, MO). Cholesterol and ergosterol were obtained from Steraloids (Wilmington, NH). Stock DHE (5 mg/mL) and cholesterol (10 mg/mL) solutions were prepared in 95% EtOH containing 1 mol % butylated hydroxytoluene (BHT) and stored at −70 °C. Metrizamide was obtained from Accurate Chemical and Scientific Corp. (Westbury, NY). Monoclonal antibodies to lysosomal membrane protein-2 (anti-LAMP-2) and to plasma membrane Na^+K^+-ATPase (anti-Na^+K^+-ATPase) were purchased from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA).

Cell Culture, Cellular Subfractionation, and Membrane Isolation. Mock transfected L-cell fibroblasts and L-cells transfected with cDNA encoding the 15 kDa pro-SCP-2 were obtained and cultured as described earlier (*38*, *65*). Lysosomal membranes were isolated from L-cell fibroblasts as described previously (*66*, *67*). Plasma membrane isolation was performed as follows: After 90 h of growth in 10% FBS Higuchi medium (*68*), the culture medium was removed, and the cells were rinsed twice with PBS. The cells were then harvested in isotonic sucrose/TRIS/EDTA (0.25 M sucrose/10 mM TRIS-HCl/1 mM EDTA, pH 7.4), centrifuged for 10 min at 1100*g* with a JA25.5 fixed angle rotor on an Avanti J25 Centrifuge (Beckman Inc., Fullerton, CA), resuspended in a known volume of isotonic sucrose/TRIS/EDTA, and counted. This cell suspension was disrupted with a N_2 bomb cell disrupter (Parr Instrument Co, Moline, IL) set at ∼40 psi of N_2 for ∼13 min. The postnuclear supernatant was collected following centrifugation at 1700*g* for 10 min with a JA25.5 fixed angle rotor on an Avanti J25 centrifuge (Beckman Inc., Fullerton, CA). This supernatant was in turn centrifuged at 15 800 rpm for 60 min using an SW28 rotor and Model UL60 ultracentrifuge (Beckman Instruments, Fullerton, CA). The pellet was resuspended in isotonic sucrose/TRIS/EDTA and placed onto a sucrose gradient at 39 000 rpm for 2 h using an SW40Ti rotor and Model XL90 ultracentrifuge (Beckman Instruments, Fullerton, CA). The discontinuous sucrose gradients consisted of the following percent sucrose in deionized water (w/v): 8.3%, 20%, 27%, 29%, 32%, 35%, 40%, and 55% sucrose. The enriched fraction of plasma membrane occurred at the 27%/29% sucrose interface. This fraction was collected and diluted with 1 mM TRIS and subsequently sedimented at 39 000 rpm on the SW40Ti rotor and XL90 ultracentrifuge. All isolations were performed with ice-cold solvents and chilled equipment. All buffer solutions were prepared using deionized water. The purity of the respective membrane fractions was determined by quantitative Western blotting

using markers for lysosomal membranes, LAMP-2 (*66*), and for plasma membranes, Na^+K^+-ATPase (*69*). Additional markers for plasma membrane purity were based on lipid composition of plasma membranes vs cell homogenate (see Results).

Lipid Extraction and Analysis. Plasma membrane lipids were extracted, resolved into phospholipids and neutral lipids, and quantitated (*55*). Neutral lipids were further resolved by high-performance liquid chromatography (HPLC) (*55*). Phospholipids were also resolved into individual components and quantitated as determined previously (*70*).

Determination of Dehydroergosterol Steady-State Fluorescence and Polarization in Lysosomal Membranes and Plasma Membranes Isolated from L-Cell Fibroblasts. Steady-state fluorescence intensity and polarization measurements of dehydroergosterol in lysosomal membranes and plasma membranes were performed as described earlier (*66*, *67*). The residual light scatter (from both donor and acceptor membranes) contribution to polarization data was corrected by converting polarization to anisotropy according to the equation $r = 2P/(3 - P)$ and subtracting the residual fluorescence anisotropy of both donor and acceptor membranes from all experimental data. Artifacts due to inner filter effects were avoided by keeping the absorbance of sample solutions at the excitation wavelength (324 nm) below 0.15. All fluorescence polarization experiments were conducted in filtered 10 mM PIPES buffer solution at pH = 7.4 in a quartz cuvette which contained 2 mL of sample and was thermostated to 37 ± 0.3 °C through use of a water heating bath (Fisher Scientific, Pittsburgh, PA).

Measurement of Intermembrane Sterol Transfer. Sterol transfer between isolated lysosomal membranes and plasma membranes was determined using a fluorescent sterol (dehydroergosterol) exchange assay previously described by our laboratory for determination of sterol transfer between other biological membranes (*65*−*67*, *71*, *72*). The basis for using dehydroergosterol as a probe for cholesterol transfer was reviewed earlier (*73*). Detailed descriptions of this assay, appropriate controls, the basis for the lack of polarization change in the absence of acceptor membranes, and justifications are provided in the above cited publications. Standard curves for dehydroergosterol in lysosomal−lysosomal membrane exchanges (*66*) and for dehydroergosterol in plasma membrane−plasma membrane exchanges (*65*, *71*) determined previously by our laboratory were used to construct new standard curves for lysosomal membrane−plasma membrane and plasma membrane−lysosomal membrane exchanges. The general procedure for constructing heterogeneous (i.e., dissimilar donor and acceptor) standard curves followed the method for other heterogeneous standard curves described previously (*65*, *67*, *71*). Briefly, plots of polarization versus mole fraction of dehydroergosterol (as a function of total membrane lipid) were constructed from these data as follows. Fluorescence polarization is described by a hyperbolic function (*74*)

$$P = P_0C/(B + C) \tag{1}$$

where P is the measured fluorescence polarization of dehydroergosterol at concentration C in the membranes, P_0 is the dehydroergosterol polarization at its infinite dilution in the membranes, and B is a constant. Because eq 1 describes the polarization−concentration relationship for dehydroergosterol in donor membranes only and the measured polarization in the assay is the result of the combined polarization of dehydroergosterol in both the donor and acceptor membranes, eq 1 was modified to incorporate the contribution of the dehydroergosterol in the acceptor membranes. The concentration of dehydroergosterol in the donor (X_d) and acceptor (X_a) membranes was then written as

$$X_d = C_d/C_t \tag{2}$$

$$X_a = 1 - X_d = 1 - (C_d/C_t) \tag{3}$$

where C_t was the concentration of dehydroergosterol at time $t = 0$ and C_d was the concentration of dehydroergosterol in the donor membrane at any later time, t. To combine the contribution of dehydroergosterol in the donor and acceptor membranes to obtain the total fluorescence polarization, polarization values were converted to anisotropy, because anisotropy (r) is an additive function while polarization is not.

$$r = 2P/(3 - P) \tag{4}$$

The calculated anisotropy of donor and acceptor membranes was then described by the equation:

$$r = f_dr_d + f_ar_a \tag{5}$$

where f_a and f_d were the fraction of dehydroergosterol in the acceptor and donor membranes, respectively, while r_a and r_d were the fluorescence anisotropies of dehydroergosterol in the acceptor and donor membranes, respectively. By combining eqs 1−5, recalling that the exchange assay is performed with a 10-fold excess of acceptor membrane, and combining several terms, the anisotropy was described in terms independent of the mole fraction of dehydroergosterol in the acceptor membranes:

$$r = r_0\{X_d/(1 + DX_d)\} + r_0\{(1 - X_d)/(1 + (D/10)(1 - X_d))\} \tag{6}$$

where r_0 was the anisotropy of dehydroergosterol at infinite membrane dilution. The constant D was related to r_0 and the constant B from eqs 1−7:

$$D = ZB(1 + (r_0/2)) \tag{7}$$

where Z was the mole fraction of dehydroergosterol in the total membrane lipid. The parameter Z was estimated by the equation:

$$Z = (P_0 - P)/PB \tag{8}$$

where P_0 and B were the parameters from eq 1 and P was the fluorescence polarization of dehydroergosterol in the donor membranes in the absence of acceptor membranes. The first and second terms in eq 5 were the contributions to the anisotropy by the donor and acceptor membranes, respectively. Fluorescence polarization was then calculated from the anisotropy by using the equation:

$$P = 3r/(2 + r) \tag{9}$$

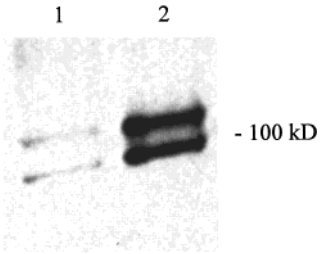

FIGURE 1: Western blot of L-cell plasma membrane Na^+,K^+-ATPase. Lanes: 1, cell homogenate; 2, isolated plasma membrane fraction. Western blotting was performed as described in Materials and Methods.

The resultant parameters used in this work were $r_0 = 0.2528$ and $D = 0.6488$ for the plasma membrane donor and were $r_0 = 0.18174$ and $D = 0.6663$ for the lysosome donor. Standard curves relating the mole fraction of dehydroergosterol in the donor membranes (X_d) to the measured fluorescence polarization were then calculated for various initial concentrations of dehydroergosterol in the donor membranes. To calculate the fraction of dehydroergosterol remaining in the donor lysosomes during an exchange assay curve, the data were best fit to a polynomial equation of the form:

$$P = \sum b_n X_d^{\,n} \qquad (10)$$

For a two-exponential fit:

$$X_d = f_1 \exp(-k_1 t) + f_2 \exp(-k_2 t) + f_3 \qquad (11)$$

where f_1 and f_2 denote exchangeable fractions, k_1 and k_2 denote exchange rates, and f_3 denotes the very slowly/nonexchangeable fraction.

For sterol exchange between lysosomal membrane donors and plasma membrane acceptors, a polynomial with three terms yielded a fit with $r^2 = 0.9999$:

$$F(x) = y(0) + b_1 X_d + b_2 X_d^{\,2} \qquad (12)$$

where $y(0) = 0.2951$, $b_1 = -0.04603$, and $b_2 = -0.09962$. Exchange between plasma membrane donors and lysosomal membrane acceptors followed the same curve described in eq 10, except that the resultant fit had $r^2 = 0.9988$, $y(0) = 0.2541$, $b_1 = 0.03122$, and $b_2 = -0.07143$.

The initial rate of dehydroergosterol exchange between lysosomal membranes was estimated from the first 10 min of exchange data by using a standard curve based on lysosomal membrane and plasma membrane sterol content and prepared similarly to those previously described for other biological membranes (*66*, *67*, *71*).

RESULTS

Enzyme and Lipid Markers for L-Cell Plasma Membrane Purification. To determine the effect of SCP-2 expression on L-cell plasma membrane lipid distribution, it was essential to obtain purified plasma membranes. The purity of the plasma membrane fraction, isolated as described in Materials and Methods, was established by use of protein and lipid markers. Western blotting with anti-Na^+K^+-ATPase revealed that this marker was present both in the purified plasma membrane fraction and in the crude homogenate (Figure 1). Quantitative analysis showed that the plasma membrane fraction was enriched >10-fold in Na^+K^+-ATPase as compared to crude homogenate. Examination of the L-cell plasma membrane lipid composition expressed as lipid mass (nanomoles per milligram of protein) revealed that two lipid fractions, phospholipids and cholesterol, comprised nearly 90% of L-cell plasma membrane lipids (Table 1). Plasma membrane phospholipids were present at 294 ± 45 nmol/mg of protein (Table 1), 2-fold enriched over cell homogenate phospholipid mass (149 ± 16 nmol/mg of protein). Cholesterol mass was enriched 6.1-fold in L-cell plasma membranes (397 ± 59 nmol/mg of protein, Table 1) as compared to crude homogenate (65 ± 9 nmol/mg of protein). The plasma membrane cholesterol/phospholipid molar ratio was 1.23 ± 0.03 mol/mol. In summary, both protein and lipid markers indicated that the L-cell plasma membrane fraction was highly purified and typical of that reported for plasma membranes (*73*, *75*, *76*). The overall purification of plasma membranes as compared to crude homogenate was up to 10-fold, i.e., 90% pure, depending on the marker used.

Table 1: Effect of SCP-2 Expression on Plasma Membrane Lipid Mass and Composition[a]

	mass (nmol/mg of protein)		composition (mol %)	
lipid class	control	SCP-2	control	SCP-2
phospholipid	294 ± 45	207 ± 25[c]	37.5 ± 5.7	40.7 ± 4.9
cholesterol	397 ± 59	248 ± 25[c]	50.7 ± 7.5	48.7 ± 4.9
triglycerides	10 ± 3	5 ± 1[b]	1.3 ± 0.4	
cholesterol ester	82 ± 11	49 ± 2[c]	10.5 ± 1.4	9.6 ± 0.3
total	783 ± 62	509 ± 32[c]		

	ratio (nmol/nmol)	
lipid class	control	SCP-2
neutral lipid/phospholipid	1.66 ± 0.10	1.45 ± 0.10
cholesterol/phospholipid	1.23 ± 0.03	1.17 ± 0.07

[a] Values represent means ± standard error, $n = 5-6$. Neutral lipid refers to cholesterol + triglycerides + cholesteryl ester. Minor quantities of glycolipids were detected but were unaltered by SCP-2 expression and represented <1% of total lipid. [b] Significant from control, $p < 0.05$. [c] Significant from control, $p < 0.01$.

Low Levels of Cholesteryl Esters and Triglycerides Detected in the Purified Plasma Membrane Fraction. The purified L-cell plasma membrane fraction contained low amounts of cholesteryl esters, 82 ± 11 nmol/mg of protein or 78 ± 10 nmol/mg of lipid (10% of total lipids) (Table 1). In addition, low levels of triglycerides, 10 ± 3 nmol/mg of protein (1% of total lipids), were also observed (Table 1). When compared to the cholesteryl ester and triglyceride content of L-cell homogenates, 23.2 ± 4.6 and 3.1 ± 0.3 ($n = 4-5$) nmol/mg of protein, respectively, L-cell plasma membranes were enriched 3.6- and 3.2-fold in cholesteryl ester and triglyceride. The plasma membrane purity obtained herein was consistent with the literature (*76*−*80*).

Expression of Sterol Carrier Protein-2 at Physiological Levels in Transfected L-Cell Fibroblasts. To determine the effect of sterol carrier protein-2 (SCP-2) expression on lipid distribution to the plasma membrane of intact cells, L-cells were transfected with the cDNA for the precursor protein, 15 kDa pro-SCP-2 (see Materials and Methods). As for almost all tissues and cells examined, the 15 kDa pro-SCP-2 was completely cleaved. Western blotting revealed that 13 kDa SCP-2 represented 0.03% of soluble protein in transfected L-cells overexpressing SCP-2 but was near the limit

of detectability in untransfected and mock-transfected L-cells (data not shown). Since this level of SCP-2 expression was in the range of that found in most animal tissues (0.08−0.01% of soluble protein), the effects of SCP-2 expression on plasma membrane lipid mass (nmol/mg of protein) and percent composition shown in the following sections were not due to expression of SCP-2 at nonphysiological levels.

Effect of Sterol Carrier Protein-2 Expression in Transfected L-Cell Fibroblasts on Plasma Membrane Purification. To determine the effect of SCP-2 expression on plasma membrane lipid distribution, it was essential that the purity of plasma membranes from SCP-2-expressing cells be similar to that of control cells. Western blotting with anti-Na^+K^+-ATPase revealed that the plasma membrane fraction was enriched >10-fold in Na^+K^+-ATPase as compared to crude homogenate (not shown). The phospholipids and cholesterol comprised nearly 90% of the plasma membrane lipids from the SCP-2-expressing cells (Table 1). Plasma membrane phospholipids were present at 207 ± 25 nmol/mg of protein (Table 1), 2.8-fold enriched over cell homogenate phospholipid mass (74 ± 12 nmol/mg of protein). Cholesterol mass was enriched 4-fold in L-cell plasma membranes (248 ± 25 nmol/mg of protein, Table 1) as compared to crude homogenate (63 ± 5 nmol/mg of protein). The SCP-2-expressing cell plasma membrane molar ratio of cholesterol/phospholipid was high, 1.17 ± 0.07 mol/mol (Table 1). Taken together, the overlapping, similar relative purification of a variety of markers for plasma membranes from SCP-2-expressing cells to that of control cells suggested that effects of SCP-2 expression on plasma membrane lipid mass (nmol/mg of protein) and percent composition were not likely to be due to differential purification of plasma membranes.

SCP-2 Expression Decreases Plasma Membrane Lipid Mass without Altering Lipid Class Composition of Transfected L-Cell Fibroblasts. Although the lipid mass and composition of plasma membranes from control cells did not differ from those of mock-transfected cells (not shown), the mass of nearly all lipid components of the plasma membrane was decreased in SCP-2-expressing cells (Table 1): total lipid decreased from 783 to 509 nmol/mg of protein (35% decrease, $p < 0.05$); phospholipids decreased from 294 to 207 nmol/mg of protein (30% decrease, $p < 0.05$); cholesterol decreased from 397 to 248 nmol/mg of protein (38% decrease, $p < 0.01$); cholesteryl ester decreased from 82 to 49 nmol/mg of protein (40% decrease, $p < 0.05$) (Table 1). Because the decrease in plasma membrane lipid mass in response to SCP-2 expression was essentially proportional among these lipid classes, the percent lipid class composition of the plasma membrane lipids was unaffected (Table 1) and there were no significant alterations in the ratios of neutral lipid/phospholipid or cholesterol/phospholipid (Table 1).

SCP-2 Expression Alters both the Mass Distribution and Composition of Phospholipid Classes in Plasma Membranes from Transfected L-Cell Fibroblasts. Since SCP-2 binds and selectively transfer phospholipids between membranes, the possibility that SCP-2 expression selectively altered the plasma membrane phospholipid class distribution was examined. The phospholipid class mass and composition of control (Table 2) and mock-transfected cells (not shown) did not differ significantly. Phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine comprised the majority (51%, 18%, and 16%, respectively) of total phospholipid mass in plasma membranes of control cells (Table 2). With the exception of phosphatidylserine, SCP-2 expression differentially altered phospholipid mass (nmol/mg of protein) of the plasma membrane phospholipid classes (Table 2): increased phosphatidylinositol by 47%, $p < 0.01$ (from 18 to 33); decreased phosphatidylcholine by 64%, $p < 0.01$ (from 144 to 53); decreased phosphatidylethanolamine by 53%, $p < 0.01$ (from 45 to 21); decreased sphingomyelin (CerPCho) by 44% (from 25 to 14). Most important, however, when these phospholipid masses were expressed in terms of percent composition, it was evident that SCP-2 differentially altered ($p < 0.05$) the relative proportion of phospholipids to allow selective retention of phosphatidylinositol and phosphatidylserine at the expense of phosphatidylcholine and phosphatidylethanolamine in the plasma membrane (Table 2). Furthermore, SCP-2 expression resulted in a nearly 3.1-fold increased ratio (from 0.32 to 0.99) of anionic/neutral charged phospholipids [i.e., (PtdSer + PtdIns)/(PtdEtn + PtdCho + CerPCho)] in the plasma membrane (Table 2). In summary, SCP-2 expression dramatically altered the phospholipid class mass and the proportion of anionic phospholipids of the phospholipid fraction of the plasma membranes. Further, SCP-2 expression resulted in selective retention and enrichment of phosphatidylinositol and phosphatidylserine in the plasma membranes. Phosphatidylinositol is an important molecule in intracellular signaling and vesicular trafficking while phosphatidylserine is an important annular lipid for plasma membrane proteins.

Table 2: Effect of SCP-2 Expression on L-Cell Plasma Membrane Phospholipid Mass and Composition[a]

phospholipid class	mass (nmol/mg of protein)		composition (mol %)	
	control	SCP-2	control	SCP-2
PtdEtn	44.5 ± 10.5	21.3 ± 1.9[c]	15.8 ± 3.7	12.1 ± 1.1[b]
PtdSer	49.8 ± 14.3	54.4 ± 2.6	17.7 ± 5.1	30.8 ± 1.5[c]
PtdIns	18.0 ± 4.1	33.4 ± 7.9[c]	6.4 ± 1.5	18.9 ± 4.4[c]
PtdCho	144.1 ± 23.4	53.4 ± 5.7[c]	51.2 ± 8.3	30.3 ± 3.2[c]
CerPCho	25.0 ± 6.5	14.1 ± 4.6[b]	8.9 ± 2.3	8.0 ± 2.6
total	281.4 ± 30.4	176.8 ± 22.6[c]		

[a] Values represent means ± standard error, $n = 4-6$. [b] Significant from control, $p < 0.05$. [c] Significant from control, $p < 0.01$.

Effect of SCP-2 Expression on the Mass and Composition of Fatty Acids Esterified to Plasma Membrane Phospholipids. Since SCP-2 binds fatty acids/fatty acyl CoAs and enhances their microsomal transacylation in vitro, the possibility that SCP-2 expression might alter the types of fatty acids esterified to plasma membrane phospholipids was examined. Nearly two dozen fatty acid species were esterified to plasma membrane phospholipids, with palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1 n-9) comprising the largest amounts by mass (Table 3). SCP-2 expression significantly decreased the mass (nmol/mg of protein) of the majority of fatty acid species comprising 91% of the plasma membrane phospholipid fatty acid species (Table 3). However, 9% of total plasma membrane phospholipid fatty acids (i.e., 14:1, 18:1 n-6, 18:3 n-3, 20:2 n-6, 22:3 n-3, 24:0, and 24:1 n-9) were selectively enriched in the plasma membrane phospholipid fraction (Table 3). When these effects on specific plasma membrane phospholipid fatty acid species were grouped into larger categories, SCP-2 expression decreased the mass of all classes (saturated, unsaturated, monounsaturated, polyunsaturated fatty acids) per milligram of protein

Table 3: Effect of SCP-2 Expression on L-Cell Plasma Membrane Phospholipid Fatty Acid Mass and Composition[a]

	mass (nmol/mg of protein)		composition (mol %)	
fatty acid	control	SCP-2	control	SCP-2
10:0	5.21 ± 0.61	2.74 ± 0.15[c]	0.38 ± 0.04	0.36 ± 0.02
12:0	14.03 ± 0.94	11.10 ± 0.52[b]	1.03 ± 0.07	1.47 ± 0.07[b]
14:0	ND[d]	1.60 ± 0.26[c]	ND	0.21 ± 0.03[c]
14:1 n-5	14.99 ± 1.19	10.33 ± 0.64	1.10 ± 0.09	1.36 ± 0.08
16:0	199.65 ± 9.85	100.97 ± 8.75[c]	14.69 ± 0.72	13.33 ± 1.16
16:1 n-7	40.91 ± 2.36	17.94 ± 1.01[c]	3.01 ± 0.17	2.37 ± 0.13[b]
18:0	310.18 ± 11.78	161.19 ± 10.28[c]	22.83 ± 0.87	21.28 ± 1.36
18:1 n-9	521.74 ± 20.05	300.10 ± 13.07[c]	38.40 ± 1.48	39.62 ± 1.73
18:1 n-6	16.71 ± 1.43	13.33 ± 0.80	1.23 ± 0.11	1.76 ± 0.11[b]
18:2 n-6	21.84 ± 0.40	11.56 ± 0.27[c]	1.61 ± 0.03	1.53 ± 0.04
18:3 n-6	26.20 ± 5.02	13.21 ± 1.99[b]	1.93 ± 0.37	1.74 ± 0.26
18:3 n-3	8.21 ± 1.59	6.67 ± 1.73	0.60 ± 0.12	0.88 ± 0.23
20:0	10.48 ± 0.63	3.88 ± 0.28[c]	0.77 ± 0.05	0.51 ± 0.04[c]
20:1 n-9	37.17 ± 0.99	22.97 ± 2.47[c]	2.74 ± 0.07	3.03 ± 0.33
20:2 n-6	13.05 ± 0.28	11.42 ± 2.30	0.96 ± 0.02	1.51 ± 0.30
20:3 n-6	5.69 ± 0.57	3.30 ± 0.21[c]	0.42 ± 0.04	0.44 ± 0.03
20:4 n-6	46.22 ± 2.84	21.29 ± 2.10[c]	3.40 ± 0.21	2.81 ± 0.28
22:0	12.63 ± 1.12	7.95 ± 0.40[c]	0.41 ± 0.06	1.05 ± 0.05[c]
22:3 n-3	13.24 ± 1.05	11.93 ± 2.47	0.97 ± 0.08	1.57 ± 0.33
22:4 n-6	4.45 ± 0.22	1.63 ± 0.12[c]	0.33 ± 0.02	0.22 ± 0.02[b]
22:6 n-3	20.25 ± 0.61	7.78 ± 0.52[c]	1.49 ± 0.05	1.03 ± 0.07[c]
24:0	14.14 ± 0.07	12.79 ± 1.53	1.04 ± 0.01	1.69 ± 0.20[b]
24:1 n-9	1.66 ± 0.25	1.86 ± 0.17	0.12 ± 0.02	0.25 ± 0.02[c]

[a] Values represent means ± standard error, $n = 3-6$. [b] Significant from control, $p < 0.05$. [c] Significant from control, $p < 0.01$. [d] ND indicates not detectable.

Table 4: Effect of SCP-2 Expression on L-Cell Plasma Membrane Phospholipid Fatty Acid Mass and Composition Ratios[a]

	mass (nmol/mg of protein)		composition (mol %)	
fatty acid	control	SCP-2	control	SCP-2
saturated/protein	566.32 ± 24.96	302.22 ± 22.18[b]	41.68 ± 1.84	39.89 ± 2.93
unsaturated/protein	792.32 ± 38.86	455.32 ± 29.85[b]	58.32 ± 2.86	60.11 ± 3.94
MUFA/protein	633.18 ± 26.27	366.53 ± 18.15[b]	46.60 ± 1.93	48.38 ± 2.40
PUFA/protein	159.14 ± 12.59	88.80 ± 11.70[b]	11.71 ± 0.93	11.72 ± 1.54

	ratios (nmol/nmol)	
fatty acid	control	SCP-2
unsaturated/saturated	1.40 ± 0.092	1.51 ± 0.148
PUFA/MUFA	0.25 ± 0.022	0.24 ± 0.034

[a] Values represent means ± standard error, $n = 3-6$. [b] Significant from control, $p < 0.01$.

in the plasma membrane phospholipids (Table 4). However, when expressed as percent composition, the expression of SCP-2 did not alter the ratios of saturated fatty acids/protein, unsaturated fatty acids/protein, monounsaturated fatty acids/protein, polyunsaturated fatty acids/protein, or unsaturated/saturated fatty acids or the ratio of polyunsaturated/monounsatured fatty acids in the plasma membrane phospholipid fatty acids (Table 4). Thus, the changes in specific fatty acids elicited by SCP-2 expression as noted in Table 3 appeared to be compensated by alterations in similar types of other fatty acids in order to maintain the above percent ratios constant.

Purification of Lysosomal Membranes from L-Cell Fibroblasts. To show that the altered plasma membrane lipid mass and composition in SCP-2-expressing cells was not due to SCP-2 directly inhibiting lysosomal lipid transfer to the plasma membrane, it was essential to demonstrate that SCP-2-mediated lipid transfer from the lysosomal membrane to the plasma membrane was not impaired. To test this possibility, SCP-2-mediated transfer of cholesterol was examined between lysosomal membranes and plasma membranes isolated from SCP-2 overexpressing L-cells and controls. The purity of the plasma membrane fraction was detailed above. The purity of the lysosomal membrane fraction was established by use of the lysosomal membrane protein-2 (LAMP-2) as a marker. Western blotting revealed that LAMP-2 was enriched 52-fold in the purified lysosomal membrane fraction as compared to the crude homogenate. Neither the presence of dehydroergosterol (used to measure sterol transfer; see Materials and Methods) nor overexpression of SCP-2 in transfected L-cells affected the relative purification of lysosomal membranes (not shown) and plasma membranes (see above).

Molecular Sterol Transfer between Lysosomal Membranes and Plasma Membranes: Construction of Standard Curves. To probe both spontaneous and protein-mediated molecular transfer of cholesterol using the fluorescent sterol exchange assay (Materials and Methods), it was first necessary to establish dehydroergosterol polarization standard curves for transfer between dissimilar acceptor and donor pairs (i.e., lysosomal membranes and plasma membranes). These standard curves quantitatively describe molecular sterol transfer between lysosomal membrane donor−plasma membrane acceptor (Figure 2A) and plasma membrane donor−lysosomal membrane acceptor (Figure 2B) pairs. At $X_d = 1$ the donor membrane contained all of the dehydroergosterol, and dehydroergosterol polarization was low in lysosomal membrane (Figure 2A) or plasma membrane (Figure 2B) donor membranes. This was consistent with the dehydroergosterol fluorescence being initially self-quenched in the donor membranes. As the exchange progressed (decreasing mole fraction of dehydroergosterol in the donor lysosomal membrane), the dehydroergosterol polarization increased. Straight lines (not shown) drawn for the fluorescence polarization between dehydroergosterol mole fractions (X_d) between 1.0 and 0.8 in both panels A and B of Figure 2 were linear ($r^2 = 0.9999$). This indicated that, for dehydroergosterol transfer from lysosomal membranes−plasma membranes as well as from plasma membranes−lysosomal membranes, the initial change in fluorescence polarization was directly proportional to the change in mole fraction of dehydroergosterol. This relationship allowed determination of the initial rate of molecular dehydroergosterol exchange between donor and acceptor membranes by comparison of polarization values to the standard curve. Separation of the donor and acceptor membranes was not required in the assay.

Spontaneous Sterol Transfer from Lysosomal Membranes to Plasma Membranes. To examine spontaneous sterol transfer, dehydroergosterol polarization in lysosomal membrane donors was monitored in the absence or presence of a 10-fold excess of acceptor plasma membranes. In the absence of plasma membrane acceptors, polarization did not significantly change over several hours (not shown). Addition of a 10-fold excess of acceptor plasma membranes resulted in slow, spontaneous sterol transfer from lysosomal membranes to plasma membranes as indicated by very little increase in dehydroergosterol polarization (Figure 3A, bottom curve). The initial rate of spontaneous molecular sterol transfer from lysosomal membranes to plasma membranes, determined

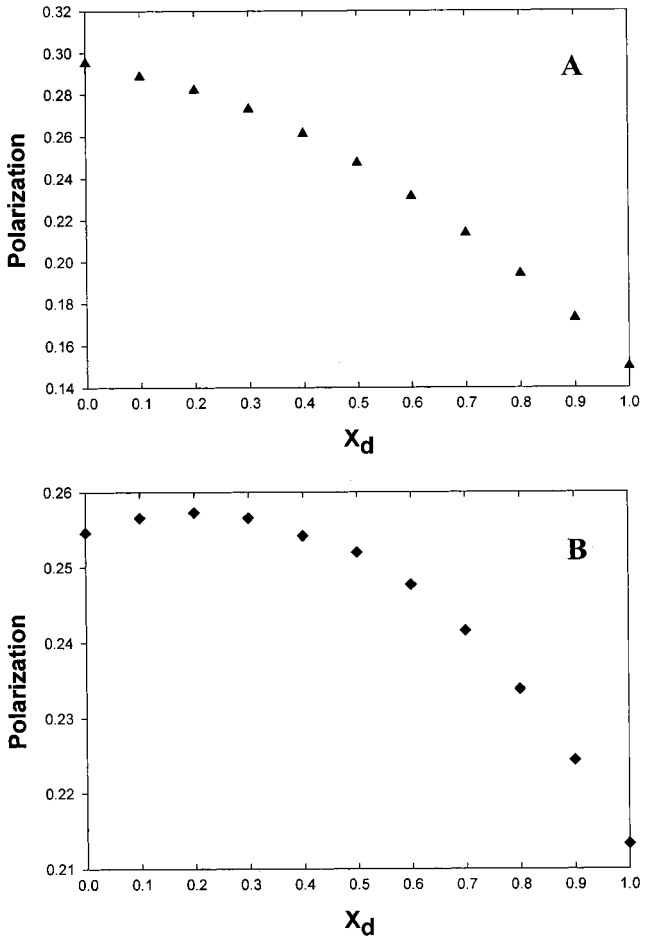

FIGURE 2: Dehydroergosterol polarization as a function of dehydroergosterol mole fraction for lysosome donor/plasma membrane acceptor sterol transfer. Panel A denotes lysosomal membrane donor and plasma membrane acceptor. Panel B denotes plasma membrane donor and lysosomal membrane acceptor.

using the standard curve in Figure 2A as described in Materials and Methods, was only 0.005 ± 0.001 pmol/min (Table 5). The half-time for this spontaneous sterol transfer from the lysosomal membrane to the plasma membrane was >4 days (Table 6). The observation of very slow spontaneous transfer of sterol from the lysosomal membrane to the plasma membrane suggested that the presence of plasma membrane acceptors alone did not induce or account for the rapid (minutes), nonvesicular component of cholesterol transfer.

Effect of SCP-2 on Transfer of Lysosomal Membrane Cholesterol to Plasma Membranes. SCP-2 did not inhibit transfer of sterol from lysosomal membranes to the plasma membrane. Instead, SCP-2 induced rapid sterol transfer as indicated by the dramatic increase in dehydroergosterol polarization (Figure 3A, top curve). The onset of this SCP-2-induced increase in sterol transfer was detectable within 2 min (Figure 3A, insert). To compare these data on dehydroergosterol transfer from lysosomal membranes to plasma membranes, obtained in vitro, with those of others using radiolabeled cholesterol in intact cells, the dehydroergosterol polarization data were converted to anisotropy (see Materials and Methods) and expressed as a percent of the maximal anisotropy (Figure 4). These data confirmed that the onset of detectable SCP-2-induced sterol transfer occurred in less than 2 min (Figure 4).

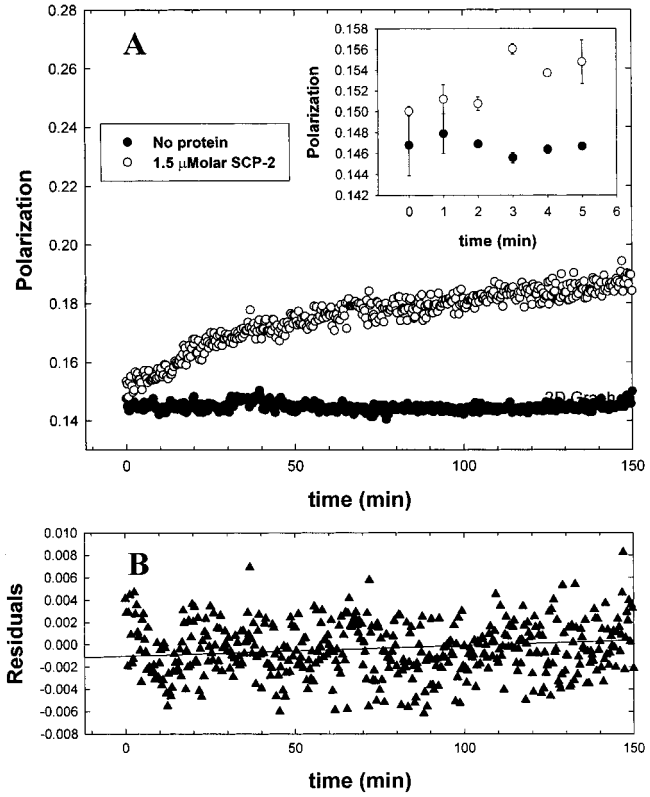

FIGURE 3: Effect of SCP-2 on sterol transfer from lysosomal membrane donor to plasma membrane acceptor membranes. In panel A, the curves show the change in fluorescence polarization as a function of time after addition of a 10-fold excess of acceptor (dehydroergosterol-deficient) plasma membranes to donor (dehydroergosterol-rich) lysosomal membranes. The inset shows a magnification of the early time points of the exchange to demonstrate the time at which the spontaneous and SCP-2-mediated exchanges diverge. Each point represents $n = 3$. Panel B shows a plot of the residual as a function of time. Each residual is the difference between the observed data minus the predicted value (obtained by the two-exponential fit of the data; see Materials and Methods). Since the residuals representing a good fit are supposed to be random, a linear fit of the residuals should yield a slope of zero or nearly equal to zero, as was the case in panel B.

Table 5: Initial Rates of Molecular Sterol Transfer between Lysosomal Membranes and Plasma Membranes: Effect of Sterol Carrier Protein-2

		initial rate (pmol/min)[a]		
	protein	experimental	fast component	slow component
LYS-PM[b]	none	0.005 ± 0.001		
	SCP-2	0.119 ± 0.013	0.270 ± 0.009	0.046 ± 0.006
PM-LYS	none	0.220 ± 0.028		
	SCP-2	8.683 ± 0.070	9.110 ± 0.050	2.398 ± 0.015

[a] Experimental refers to the initial rate without resolving into fast and slow components. The fast and slow component rates were calculated after fitting the exchange curves to multiple exponentials as described in Materials and Methods and Figure 8. SCP-2-mediated, but not spontaneous, exchange curves fit two components. Values refer to mean ± SD, $n = 5-6$. [b] LYS, lysosomal membranes; PM, plasma membranes. In each complementary pair, the first term refers to the donor (DHE-rich) membranes and the second term refers to the acceptor (no DHE) membranes. Units are per milliliter of medium.

By use of the appropriate standard curves as described in Materials and Methods, the initial rates of SCP-2-mediated dehydroergosterol polarization change were converted to initial rates of molecular sterol transfer. SCP-2 increased the

Table 6: Kinetic Multiexponential Analysis of Sterol Exchange between Lysosomal Membranes and Plasma Membranes: Effect of SCP-2[a]

	protein	$^1t_{1/2}$	$^2t_{1/2}$	f_1	f_2	f_3
LYS-PM	none		>4 days			1.000
	SCP-2	15.8 ± 2.2	178.5 ± 10.5	0.078 ± 0.009	0.139 ± 0.032	0.783 ± 0.014
PM-LYS	none		>4 days			1.000
	SCP-2	7.3 ± 1.1	182.4 ± 19.2	0.037 ± 0.005	0.252 ± 0.009	0.711 ± 0.010

[a] Unless otherwise stated, half-times $^1t_{1/2}$ and $^2t_{1/2}$ were in minutes. f_1 and f_2 represent the fractions due to the respective exchangeable components while f_3 represents the fraction of nonexchangeable component. Values represent the mean + SD, $n = 5-6$.

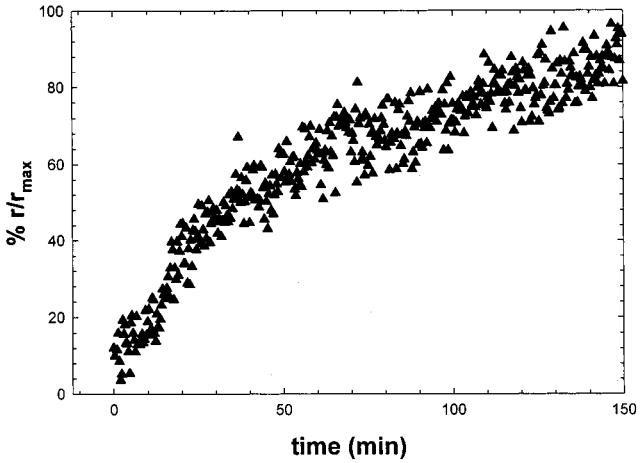

FIGURE 4: Kinetics of SCP-2-mediated sterol transfer from lysosomal membranes to donor membranes expressed as percent of maximal anisotropy change. Dehydroergosterol polarization data for SCP-2-mediated sterol transfer in Figure 3 were converted to anisotropy expressed as a percent of maximal anisotropy (% r/r_{max}) over the time period examined.

initial rate of molecular sterol transfer from lysosomal donor membranes to plasma membranes 24-fold (Table 5). SCP-2 enhanced lysosomal membrane sterol transfer to plasma membranes over a wide concentration range similar to that observed physiologically (Figure 5A, 0.1−3.0 μM), equivalent to 0.01−0.60 μg of SCP-2/$\mu\gamma$ of membrane. SCP-2 stimulation of the initial rates of molecular sterol transfer from lysosomal membranes to plasma membranes was saturable as a function of SCP-2 concentration (Figure 5A). A Lineweaver−Burk plot of these data (Figure 6A) was linear ($r^2 = 0.9952$) and showed (i) a K_m for SCP-2 of 1.429 μM and (ii) a limiting initial rate of sterol transfer induced by SCP-2 of 0.136 pmol/min, 27-fold higher than spontaneous sterol transfer from lysosomal membranes to plasma membranes.

Spontaneous Transfer of Sterol in the Reverse Direction: Plasma Membranes to Lysosomal Membranes. If sterol transfer in the reverse direction (i.e., from the plasma membrane to the lysosomal membrane) were faster than transfer from the lysosomal membrane to the plasma membrane, then this might shift the equilibrium distribution of cholesterol away from the plasma membrane. This possibility was tested as follows: In the absence of lysosomal membrane acceptors, dehydroergosterol polarization in plasma membrane donors did not significantly change over several hours (not shown). Even in the presence of a 10-fold excess of acceptor lysosomal membranes, spontaneous sterol transfer from plasma membranes to lysosomal membranes was slow (Figure 7A, bottom curve). The initial rate of spontaneous molecular sterol transfer from plasma membranes to lysosomal membranes, determined as described in Materials and

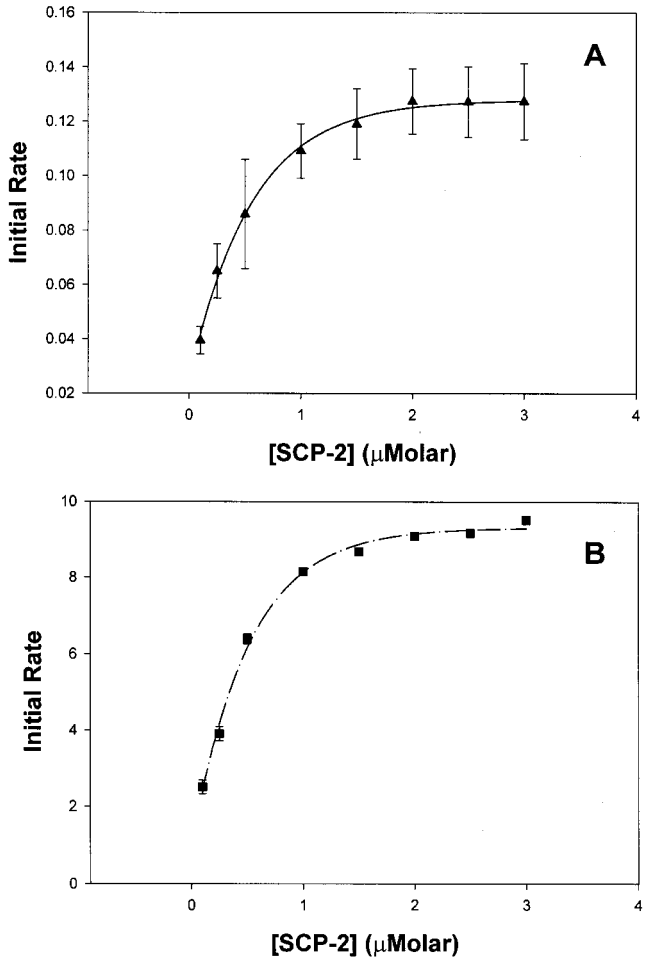

FIGURE 5: Dose−response curve for the SCP-2-mediated sterol transfer between lysosomal membranes and plasma membranes. The graphs show the initial rate (in units of picomoles per minute) as a function of SCP-2 concentration (in units of micromoles per liter). (A) Dose−response curve for SCP-2-mediated lysosomal membrane sterol transfer to plasma membranes. (B) Dose−response curve for the SCP-2-mediated sterol transfer from plasma membranes to lysosomal membranes.

Methods and using the standard curve shown in Figure 2B, was 0.220 ± 0.028 pmol/min (Table 5) while the half-time of transfer was >4 days (Table 6). This initial rate of spontaneous sterol transfer was 44-fold faster than that observed for spontaneous sterol transfer in the opposite direction from lysosomal membranes−plasma membranes (Table 1). In summary, these data are consistent with slow, bidirectional, spontaneous sterol transfer between lysosomes and plasma membranes. Together with the observation that the plasma membrane (Table 1) has severalfold higher cholesterol content than the lysosomal membranes, these data indicated that spontaneous transfer of sterol between the plasma membranes and lysosomal membranes, albeit slow,

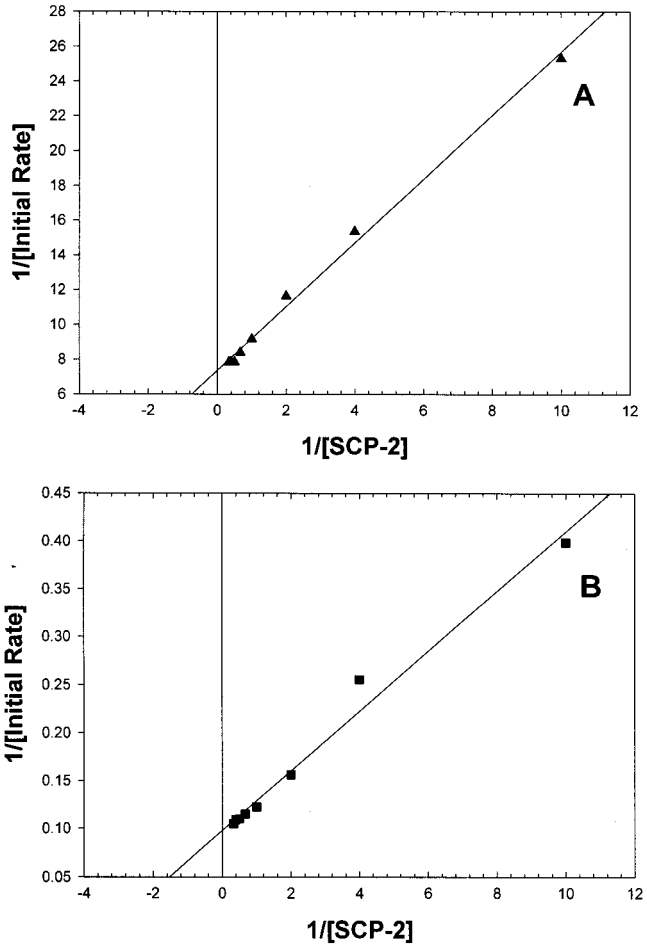

FIGURE 6: Lineweaver−Burk analysis of the SCP-2 concentration dependence of sterol transfer. The data in Figure 5 were displayed as Lineweaver−Burk plots showing the reciprocal initial rate (in units of minutes per picomole) as a function of reciprocal SCP-2 concentration (in units of liters per micromoles). (A) SCP-2-mediated sterol transfer from lysosomal membrane donor to plasma membrane acceptor. K_m = 1.429 μM and limiting initial rate = 0.136 pmol/min. (B) SCP-2-mediated sterol transfer from plasma membrane donor to lysosomal membrane acceptor. K_m = 0.671 μM and limiting initial rate = 10.22 pmol/min.

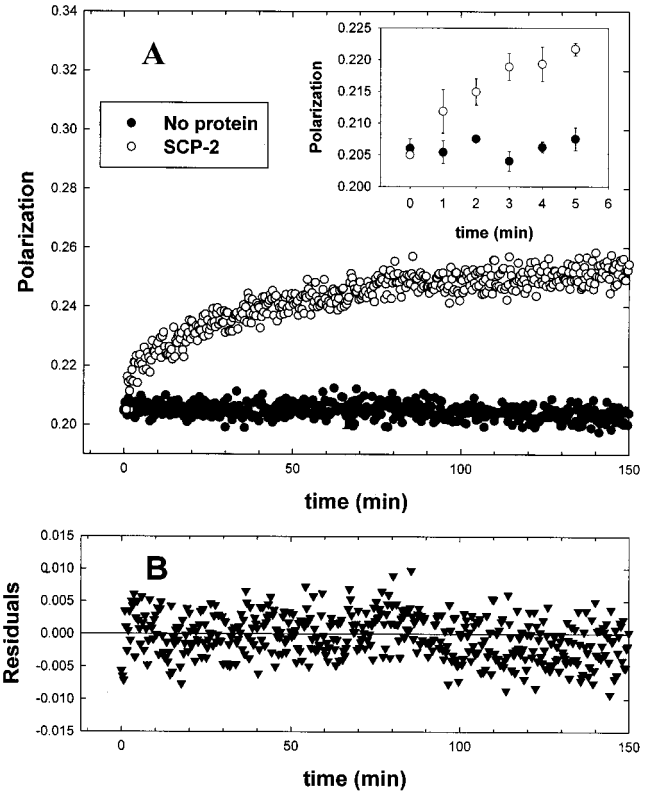

FIGURE 7: Effect of SCP-2 on sterol transfer from the plasma membrane donor to lysosomal acceptor membranes. In panel A, the curves show the change in fluorescence polarization as a function of time after addition of a 10-fold excess of acceptor (dehydroergosterol-deficient) lysosomal membranes to donor (dehydroergosterol-rich) plasma membranes. The inset shows a magnification of the early time points of the exchange to demonstrate the time at which the spontaneous and SCP-2-mediated exchanges diverge. Each point represents n = 3. Panel B shows a plot of the residual as a function of time. Each residual is the difference between the observed data minus the predicted value (obtained by the two-exponential fit of the data; see Materials and Methods). Since the residuals representing a good fit are supposed to be random, a linear fit of the residuals should yield a slope of zero or nearly equal to zero, as was the case in panel B.

is unlikely to contribute to the observed higher cholesterol content of plasma membranes.

Effect of SCP-2 on Reverse Sterol Transfer from Plasma Membranes to Lysosomal Membranes. When plasma membrane cholesterol transfer to the lysosomal membrane was probed with SCP-2, the dehydroergosterol polarization increased very rapidly (Figure 7A, top curve), with onset of sterol transfer detectable by 1 min (Figure 7A, insert). SCP-2 increased the initial rate of molecular sterol transfer from plasma membrane donors to lysosomal acceptor membranes nearly 40-fold (Table 5). The stimulatory effect of SCP-2 on sterol transfer from plasma membranes to lysosomal membranes occurred over a wide SCP-2 concentration range and was saturable with increasing SCP-2 (Figure 5B). A Lineweaver−Burk plot of these data (r^2 = 0.9830) showed a K_m for SCP-2 of 0.671 μM (Figure 6B). Furthermore, the limiting initial rate of SCP-2-mediated sterol transfer from plasma membranes, 10.22 pmol/min (Figure 6B), was 46-fold higher than spontaneous sterol transfer from plasma membranes to lysosomal membranes (Table 6). In summary, SCP-2 dramatically enhanced bidirectional sterol transfer between plasma membranes and lysosomal membranes. While the fold enhancement and K_m in the two directions differed less than 2-fold, the maximal SCP-2-mediated initial rate of sterol transfer favored sterol transfer away from the plasma membrane nearly 75-fold over sterol transfer to the plasma membrane from lysosomes, consistent with intact cell data (Table 1) showing that at equilibrium the plasma membrane cholesterol content was reduced in transfected cells overexpressing SCP-2.

Effect of SCP-2 on Sterol Transfer Dynamics between Lysosomal Membranes and Plasma Membranes: Role of Exchangeable and Nonexchangeable Domains. As indicated in Table 6, the spontaneous molecular sterol transfer between lysosomal membranes and plasma membranes was too slow to fit it to multiple exponentials. Instead, the spontaneous transfer of sterol from lysosomal membranes to plasma membranes, as well as transfer in the opposite direction, best fit a single exponential with half-time >4 days, comprising 100% of the total sterol (Table 6). Therefore, in the absence of SCP-2 these sterol pools/domains were designated as essentially nonexchangeable. In contrast, SCP-2 induced formation of rapidly exchangeable domains in the lysosomal

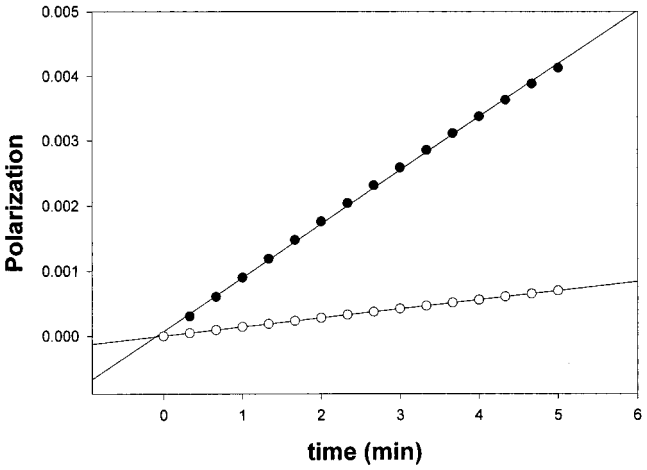

FIGURE 8: Resolving the individual contributions of the fast exchangeable and slower exchangeable components to the polarization signal during sterol transfer from lysosomal membranes to plasma membranes. The polarization data from a two-exponential fit of a typical lysosomal donor−plasma membrane acceptor + 1.5 μM exchange curve were resolved as described in Materials and Methods. The polarizations of the individual rapidly (solid circles) and slower (open circles) exchangeable components were plotted for the first 6 min of exchange. For clarity, only 1 out of 10 data points was plotted. The nonexchangeable component (not shown) remained at baseline.

membrane donor−plasma membrane acceptor pairs. In the presence of SCP-2, the lysosomal membrane donor−plasma membrane acceptor exchange curves were best described by a fit involving eqs 10−12 demonstrating two-exponential kinetics of sterol exchange. The average r^2 for lysosomal membrane donor/plasma membrane acceptor exchanges was 0.956 ($n = 5$). These values reflect the presence of two exchangeable (one rapid, one slower) sterol domains in addition to the essentially nonexchangeable domain. The rapidly exchangeable domain exhibited a $^1t_{1/2} = 15.8 \pm 2.2$ min and fraction 0.078 ± 0.009, whereas the slower exchangeable domain had $^2t_{1/2} = 178.5 \pm 10.5$ min and fraction 0.139 ± 0.032 (Table 6). Interestingly, when the contributions of these two domains to the total polarization were resolved and plotted as a function of time, two essentially linear curves were obtained over the first 6 min of exchange (Figure 8). The initial rates of molecular sterol transfer calculated from the rapidly and slower exchangeable sterol curves were 2.27- and 0.38-fold, respectively, of that for the overall (unresolved) initial rate (Table 5). Furthermore, the SCP-2-induced sterol exchange curves exhibited significant differences in polarization within 30 s for the fast exchangeable component and in <2 min for the slower exchangeable component (Figure 8).

Likewise, in the presence of SCP-2, the plasma membrane donor−lysosomal membrane acceptor exchange curves were best described by a fit involving eqs 10−12 demonstrating two-exponential kinetics of sterol exchange. The average r^2 for plasma membrane donor/lysosomal membrane acceptor exchanges was 0.985 ($n = 5$). Like the lysosomal membrane donor−plasma membrane acceptor plus SCP-2 exchange curves, these exchanges reflect the presence of two exchangeable (one rapid, one slower) sterol domains in addition to the essentially nonexchangeable domain. The rapidly exchangeable domain exhibited a $^1t_{1/2} = 7.30 \pm 1.1$ min and fraction 0.037 ± 0.006, whereas the slower exchangeable domain had $^2t_{1/2} = 182.4 \pm 9.2$ min and fraction 0.252 ± 0.009 (Table 6).

In summary, SCP-2 enhanced the bidirectional molecular transfer of sterol between lysosomal membranes and plasma membranes by inducing the formation of two exchangeable domains: a rapidly exchangeable domain ($t_{1/2} = 7-15$ min) that accounted for 4−8% of total sterol and a slower exchangeable domain ($t_{1/2}$ near 180 min) that accounted for 14−25% of total sterol. These observations were consistent with rapid sterol dynamics occurring through only a small portion of total membrane sterol in lysosomal and plasma membrane donor−acceptor pairs. When the contributions of the individual domains to the sterol transfer were resolved, detectable sterol transfer from lysosomal membranes to plasma membranes was observed within 30 s after addition of SCP-2.

DISCUSSION

It is now recognized that both lysosomal and plasma membrane lipids are organized in domains (reviewed in refs *19*, *66*, *73*, and *81*). Some plasma membrane regions (e.g., clathrin coated pits) represent domains involved with intracellular lipid trafficking primarily by vesicular mechanisms (reviewed in refs *1* and *82*). Other plasma membrane regions rich in cholesterol, anionic phospholipid, and glycosphingolipid interact with a complex set of proteins (caveolin, flotillin, etc.) to produce caveolae, rafts, and other domain structures. These domains appear to represent plasma membrane sites through which cholesterol, cholesterol esters, and phospholipids as well as other lipids rapidly (seconds to minutes) traffic by nonvesicular mechanisms (reviewed in refs *2*, *4*, *83*, and *84*). Despite these important insights regarding contributions of both vesicular and nonvesicular pathways to intracellular transport of lipids through the plasma membrane, almost nothing is known regarding either (i) the intracellular factors that regulate plasma membrane lipid dynamics or (ii) whether these factors actually alter the distribution of lipids to the plasma membrane. The work presented herein yielded several new insights supporting this possibility.

First, the data presented herein show for the first time that expression of SCP-2 altered the total lipid content of the plasma membrane in intact cells. SCP-2 decreased the total distribution of lipid (nanomoles of lipid per milligram of protein) to the plasma membrane by 35%. This effect was consistent with observations that SCP-2 expression significantly inhibited the intracellular transfer of NBD-cholesterol to the plasma membrane for efflux to HDL (*40*). Since the transfected L-cells expressing SCP-2 grew normally, it would appear that the L-cell plasma membrane can accommodate such changes. This conclusion was supported by earlier data with transfected L-cells overexpressing L-FABP, another protein that also has broad ligand distribution, the ability to transfer these ligands, and whose subcellular distribution also places it in position for altering plasma membrane lipid distribution (*52*, *85*, *86*). Thus, expression of intracellular lipid binding/transfer proteins such as SCP-2 and L-FABP significantly alters the distribution of lipids to the plasma membrane.

Second, SCP-2 reduced the distribution of cholesterol to the plasma membrane in intact cells. SCP-2 expression in

transfected cells decreased the plasma membrane content of cholesterol by 38% (present data) and enhanced cholesterol transfer away from the plasma membrane and toward to the endoplasmic reticulum for esterification by ACAT (*55*). SCP-2 expression in intact cells significantly reduced HDL-mediated efflux of cholesterol through plasma membrane caveolae (*40*). Treatment of human fibroblasts with antisense cDNA to SCP-2 abolished the rapid, but not the slower vesicular, component of intracellular sterol transfer (*56*). In summary, SCP-2 expression altered the plasma membrane distribution of cholesterol, a molecule whose homeostasic balance is essential in the cells and tissues of mammals (*2*).

Third, the time frame of the effect of SCP-2 to mediate cholesterol transfer between lysosomal membranes and plasma membrane in intact cells was modeled in vitro. SCP-2-mediated transfer of cholesterol between these organelles in vitro was detectable within 30 s−2 min and exhibited a half-time of 15 min. These kinetics were very similar to those reported for the transfer of radiolabeled cholesterol from lysosomes to plasma membranes (*87*) and from lipid droplets to plasma membranes (*40*) in intact cells.

Fourth, the directionality of SCP-2-mediated cholesterol transfer between lysosomal membranes and plasma membrane in intact cells was modeled in vitro. The in vitro SCP-2-mediated sterol transfer was more efficient down the cholesterol gradient (from the plasma membrane and toward lysosomal membrane) (Table 5) and from the plasma membrane toward the endoplasmic reticulum (*71*). Similar effects, in both intact cells and in vitro, on cholesterol distribution to the plasma membrane have been reported for another intracellular, cholesterol binding protein (L-FABP) (*47*, *52*). SCP-2 (Table 6) and L-FABP (*88*) both dramatically altered the domain structure of cholesterol in plasma membranes in vitro by creating the formation of rapidly exchangeable sterol domains, without significantly altering the cellular level of caveolin (*40*). Since the size of the lipid transfer protein-induced, rapidly exchangeable sterol domain (3−8%) was significantly greater than that of caveolae (estimated near 1%) (*4*), this was consistent with formation of additional plasma membrane microdomains. Although the exact relationship of these domains to plasma membrane caveolae remains to be determined, these domains may favor cholesterol transfer away from the plasma membranes in contrast to caveolae which favor cholesterol transfer to the plasma membrane for efflux to HDL (reviewed in ref *4*).

Fifth, the rapid in vitro SCP-2-mediated sterol transfer is consistent with at least a part of lysosomal cholesterol transfer to the plasma membrane being nonvesicular (*19*, *89*, *90*). This possibility is further supported by intact cell data showing that lysosomal cholesterol transfer to the plasma membrane was not inhibited by agents that inhibit vesicular transfer (*91*, *92*).

Sixth, SCP-2 expression reduced the distribution of phospholipid to the plasma membrane to a degree similar to that of cholesterol. SCP-2 expression decreased the plasma membrane content of phospholipid to the same extent as cholesterol such that the ratio of cholesterol/phospholipid was maintained constant. This effect of SCP-2 expression was in contrast to that of L-FABP expression which did not reduce the plasma membrane phospholipid content (*52*). Because L-FABP expression (but not SCP-2 expression) reduced the plasma membrane cholesterol/phospholipid ratio by 50%, this resulted in increased plasma membrane fluidity and decreased plasma membrane Na^+,K^+-ATPase activity, but did not alter cell viability or doubling time (*52*, *85*). These data represent the first demonstration that a intracellular protein (SCP-2) can significantly alter the phospholipid distribution to the plasma membrane.

Seventh, the effect of SCP-2 expression on phospholipid distribution to the plasma membrane was specific, resulting in selective retention of anionic phospholipids. While the mass of neutral zwitterionic phospholipid (phosphatidylcholine, sphingomyelin, phosphatidylethanolamine) species was reduced in the plasma membrane of SCP-2-expressing cells, the anionic (negatively charged) phospholipids (phosphatidylinositol, phosphatidylserine) were selectively retained. In contrast, L-FABP expression did not result in selective retention/enrichment of anionic phospholipids in the plasma membrane (*52*). While SCP-2 binds both neutral zwitterionic and anionic phospholipids, there is no competition between anionic phospholipid (phosphatidylinositol) and neutral zwitterionic phospholipid (phosphatidylcholine) binding (*93*). The mechanism whereby SCP-2 expression results in selective retention of anionic phospholipids and specifically increases phosphatidylinositol mass in the plasma membrane is not yet clear. Plasma membrane caveolae are rich in phosphatidylinositol and contain 50% of plasma membrane PIP_2 (*4*). Thus, the presence in caveolae of protein−lipid structures that bind phosphatidylinositol-containing lipids with high affinity may account for retention of phosphatidylinositol but not the increase in phosphatidylinositol mass observed in plasma membranes of SCP-2-expressing cells (Table 2). However, two other mechanisms may explain the latter observation. (i) Bovine and rat liver SCP-2 directly enhance the molecular transfer of phosphatidylinositol between membranes up to 7-fold faster than that of phosphatidylcholine (*93*, *94*). [It should be noted that this transfer specificity was not exhibited by goat liver and hepatoma SCP-2's (*95*, *96*).] In the presence of a higher level of plasma membrane proteins with high affinity for phosphatidylinositol as compared to intracellular membranes, SCP-2 may enhance the transfer and shift the equilibrium distribution phosphatidylinositol to the plasma membrane. Alternately, SCP-2 may indirectly influence vesicular transfer of phosphatidylinositol from the Golgi to the plasma membrane through its ability to bind ligands (phosphatidylinositol, fatty acyl CoA) that influence vesicular trafficking (reviewed in refs *97*−*100*). The recent observation that SCP-2 expression in L-cells enhances rapid molecular transfer of lipid while inhibiting slower vesicular transfer (*40*) favors the former possibility of SCP-2-mediated molecular phosphatidylinositol transfer. It should be noted that another phosphatidylinositol transfer protein (PITP) also enhances phosphatidylinositol transfer from the Golgi to the plasma membrane (reviewed in refs *97*−*100*). The mechanism whereby PITP enhances this transfer and equilibrium distribution of phosphatidylinositol to the plasma membrane is also not yet clear. Both molecular and vesicular PITP-mediated phosphatidylinositol transfer processes have been proposed (reviewed in refs *97*−*100*).

Eighth, SCP-2 significantly reduced the plasma membrane cholesteryl ester pool in intact, transfected cells expressing SCP-2. While plasma membranes of L-cells as well as most other cells contain small amounts of cholesteryl esters (*76*−*79*), in some cell types cholesteryl esters account for up to

33% of plasma membrane sterol (*80*). Thus, the presence of low levels of cholesteryl esters in L-cell plasma membranes was not an artifact. On the contrary, the plasma membrane cholesteryl ester pool is very important in mediating selective cholesteryl ester uptake via HDL and the plasma membrane scavenger receptor B1 (*7*, *101*−*103*). In fact, >80% of plasma membrane cholesteryl ester has been reported to be associated with caveolae, a highly specialized plasma membrane microdomain (*103*). Since SCP-2 expression in intact transfected cells reduced the plasma membrane cholesteryl ester pool by 25% ($p < 0.05$), this would suggest that SCP-2 expression altered the flux of cholesteryl ester as well as that of cholesterol (see above) through the caveolar−HDL-receptor mediated pathway.

In summary, the results presented herein demonstrated for the first time that SCP-2 significantly altered the plasma membrane lipid distribution in intact, transfected cells expressing SCP-2. SCP-2 expression reduced the plasma membrane content of cholesterol and other lipids but selectively retained phosphatidylinositol. Taken together with SCP-2's known ability to bind and transfer phosphatidylinositides, the latter result suggested that SCP-2 may also be involved in vesicular trafficking mediated by polyphosphatidylinositide signaling. The properties of lysosomal sterol transfer to isolated plasma membranes in vitro were rapid (seconds to minutes), consistent with earlier findings in intact cells suggesting contributions from a nonvesicular pathway (*91*). Interestingly, resolution of the in vitro SCP-2-mediated sterol transfer into kinetic domains yielded multiple half-times that overlapped with those typically thought of as vesicular in intact cells (*87*, *92*). The data were consistent with the plasma membrane representing an intermediate compartment through which cholesterol rapidly traffics either to other organelles within the cell or to extracellular acceptors such as HDL. It is interesting to note that SCP-2 may serve as an important contributor to retention of cholesterol within intracellular sites. This observation is supported by (i) reduced plasma membrane cholesterol mass in SCP-2 expressing cells, (ii) much higher in vitro SCP-2-mediated sterol transfer out of the plasma membrane to intracellular membranes than in the opposite direction, (iii) SCP-2 expression accelerating the transfer of plasma membrane cholesterol to the endoplasmic reticulum for esterification in intact transfected cells (*39*) and in vitro (*104*, *105*), and (iv) SCP-2 expression inhibiting the overall transfer of intracellular sterol to the plasma membrane for efflux to HDL (*40*). Although it is not known whether SCP-2 expression affects the formation of lipid rafts in the Golgi, these observations nevertheless suggest that SCP-2 may be an important contributor to cellular homeostasis of cholesterol and/or other lipids. While cholesterol enters cells primarily by the LDL-receptor-mediated pathway and leaves the cell primarily by the HDL-receptor-mediated pathway, SCP-2 appears to affect intracellular retention of cholesterol. Retention of cholesterol and/or mobilizing cholesterol from the plasma membrane or other sources is especially important for steroidogenic cells (*2*, *106*, *107*).

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