Spontaneous and Protein-mediated Sterol Transfer between Intracellular Membranes*

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Andrey Frolov†, Judith K. Woodford‡, Eric J. Murphy§, Jeffrey T. Billheimer¶, and Friedhelm Schroeder¶;

From the †Department of Physiology and Pharmacology, Texas A & M University, TVMC, College Station, Texas 77843-4466, §Andrew Jergens Company, Cincinnati, Ohio 45214-1773, and the ¶Cardiovascular Department, DuPont Merck Pharmaceutical Company, Wilmington, Delaware 19898-0400

Relatively little is known regarding intracellular cholesterol trafficking pathways. To resolve some of these potential pathways, spontaneous and protein-mediated sterol transfer was examined between different donor-acceptor membrane pairs in vitro using L-cell fibroblast plasma membrane (PM) and microsomal (MICRO) and mitochondrial (MITO) membranes. Several new exciting insights were provided. First, the initial rate of spontaneous molecular sterol transfer was more dependent on the type of acceptor than donor membrane, i.e. spontaneous intracellular sterol trafficking was vectorial. Therefore, the rate of sterol desorption from the donor membrane was not necessarily the rate-limiting step in molecular sterol transfer. Second, the rate of molecular sterol transfer was not obligatorily correlated with the direction of the cholesterol gradient. For example, although PM had a 3.2-fold higher cholesterol/phospholipid ratio than MITO, spontaneous sterol transfer was 4-5-fold faster up (MITO to PM) rather than down (PM to MITO) the concentration gradient. Third, sterol carrier protein-2 differentially stimulated the initial rate of sterol transfer for all donor-acceptor combinations, being most effective with PM donors: PM-MICRO, 27-fold; and PM-MITO, 12-fold. Sterol carrier protein-2 was less effective in enhancing sterol transfer in the reverse direction, i.e. MICRO-PM and MITO-PM (5- and 4-fold, respectively). Fourth, liver fatty acid-binding protein was limited in stimulating the initial rate of sterol transfer from PM to PM (1.5-fold), from PM to MITO (3-fold), and from MICRO to MITO (3-fold). In summary, these observations present important insights into potential sterol trafficking pathways between the major membrane components of the cell.

Sterols are essential components serving both structural and functional roles in mammalian cells. Sterols modulate membrane fluidity and thickness, thereby contributing to membrane passive permeability and activity of integral membrane proteins (1, 2). Sterols form both lateral and transbilayer sterol domains (for reviews, see Refs. 3–5), thereby providing a basis for unique membrane lipid-protein architecture. Sterols are required for cell growth (6, 7) and for cell differentiation (8). Furthermore, there appears to be synergism between cholesterol biosynthesis and synthesis of DNA, RNA, and phospholipids (9, 10). In addition, cholesterol biosynthetic intermediates such as isoprenoids are required for glycoprotein synthesis and the synthesis of ubiquinones (11, 12).

Cholesterol is not uniformly distributed in cells. Sterol synthetic activity in eukaryotic cells is concentrated in the relatively cholesterol-poor endoplasmic reticulum and peroxisomes (12), whereas the plasma membrane accommodates the major part of intracellular sterol content (13, 14). Corticosteroid synthesis from cholesterol occurs in the cholesterol-deficient inner mitochondrial membrane (15). Consequently, extensive sterol transfer and targeting must occur between these intracellular membranes, either against or with the cholesterol gradient, respectively (4, 16–18). Despite their importance to sterol homeostasis in the cell, the major pathways of intracellular sterol trafficking are still unclear.

The vast majority of information on sterol transfer/exchange has focused on model membranes (4, 16–18). With regard to biological membranes, what little is known relates to erythrocytes (for review, see Refs. 19 and 20) and cell-surface membranes (4, 21, 22). Pathways of sterol exchange between intracellular membranes (microsomes, mitochondria, etc.) remain unresolved. Thus, the first objective of this study was to explore spontaneous sterol trafficking between cellular components such as plasma, microsomal, and mitochondrial membranes. The second objective was to investigate the role of SCP-2 and L-FABP, cytosolic lipid transfer proteins, in targeting potential pathways of intracellular sterol trafficking. These specialized cytosolic proteins modulate specific reactions of sterol metabolism in vitro (23–25) and enhance sterol transfer between model membranes (25–29) and cell plasma membranes in vitro (4, 22).

EXPERIMENTAL PROCEDURES

Materials—Human recombinant SCP-2 and rat recombinant L-FABP were isolated as described (30, 31). Dehydroepiandrosterone (DHE) was synthesized and purified (32, 33). Sterol standards were from Steraloids, Inc. (Wilmington, NH) and used without further purification. All other chemicals were reagent-grade or better.

Cell lines and Membrane isolation—Mouse L-cells (L aprt tk−), from Dr. David Chaplin, Washington University, St. Louis, MO, were grown in Higuchi modified medium (14). The cells were subcultured, and stock DHE (5 mg/ml, 95% non-denatured grain ethanol) was added to the medium to obtain a final concentration of 20 μg/ml medium. For the exchange assay standard curve, the final medium DHE concentrations were varied from 0 to 40 μg/ml. The total ethanol added was held

The abbreviations used are: SCP-2, sterol carrier protein-2; L-FABP, liver fatty acid-binding protein; DHE, dehydroepiandrosterone (Δ5,7,9,11,12-18-deoxycholestan-3β-d); HPLC, high performance liquid chromatography; PIPES, piperazine-N,N′-bis(2-ethanesulfonic acid); PM, plasma membrane(s); MICRO, microsomal membrane(s); MITO, mitochondrial membrane(s).

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constant for each dish and did not exceed 0.25% (v/v). Supplementation of fibroblasts for 3 days with DHE over a wide concentration range (1–20 μg/ml medium) did not affect cell doubling time or maximal cell density (34). After 3 days of growth, the cells were harvested for isolation of plasma membrane, microsomal, and mitochondrial enriched fractions (14). Protein was determined (34, 35), and relative purity of individual fractions was monitored by specific enzyme markers (14, 22, 34, 36). In the plasma membrane enriched fraction, the specific activity of (Na⁺,K⁺)-ATPase was increased 8.3-fold, while the specific activities of microsomal marker iminodiacetate phosphate and mitochondrial marker succinate-dependent cytochrome c reductase were decreased 4.2- and 100-fold, respectively. In the microsomal fraction, the specific activity of iminodiacetate phosphate was increased 5.3-fold, while in the mitochondrial enriched fraction, the specific activity of succinate-dependent cytochrome c reductase was increased 5-fold. Based on the total amount of protein in each membrane fraction and the specific activity of the above marker enzymes, the purity of each membrane fraction was estimated near 90%. Supplementation of the fibroblasts with dehydroergosterol did not alter the specific activities of the membrane enzymes tested; (Na⁺,K⁺)-ATPase, NADPH-dependent cytochrome c reductase, and succinate-dependent cytochrome c reductase (34). The sterol/phospholipid molar ratios were 0.54, 0.24, and 0.17 in the plasma, microsomal, and mitochondrial membrane fractions, respectively. Depending on the amount of dehydroergosterol used to supplement the medium, fibroblast membranes accumulated dehydroergosterol such that up to 95, 98, and 81% of endogenous sterol can be replaced in plasma membranes, microsomes, and mitochondria, respectively (34). Specific replacements are indicated in the figure legends. Dehydroergosterol codistributed with endogenous membrane sterol, and dehydroergosterol supplementation did not affect the fibroblast individual membrane sterol/phospholipid ratio, phospholipid composition, or fatty acid composition (34).

Mitochondrial Lipid Extraction and Quantitation—Lipids were extracted in n-hexane-2-propanol (3.2, v/v), evaporated to dryness, redissolved in CHCl₃, and applied to preactivated 2.54-cm silicic acid columns. The neutral lipid fraction was eluted with 4 column volumes of CHCl₃. The phospholipid fraction was eluted using 4 column volumes of MeOH and applied to preactivated 2.54-cm silicic acid column (Alltech Associates, Inc., Deerfield, IL) in the T-format with the emission monochromator (spectral slit width of 8 nm). To reduce the spontaneous rate of sterol transfer from sterol-rich PM to sterol-depleted MITO, donor and acceptor membranes are physically separated at different time points in the assay (20, 40).

In this investigation, all exchanges were performed at 37°C. Donor membranes (14 μg of protein), obtained from cells grown in the presence of DHE, were resuspended in 2 ml of prefiltered 10 mM PIPES buffer, pH 7.4. The base-line polarization was determined over a 10-min period before a 10-fold excess of acceptor membranes (obtained from cells grown in the absence of DHE) was added to initiate exchange. Neither SCP-2 nor L-FABP altered DHE polarization in microsomal or mitochondrial donors in the absence of acceptor membranes, consistent with earlier findings for model membrane (40, 42) and plasma membrane (22) donors. Polarization was measured and averaged over 20-s intervals. Initial rates of polarization change were calculated for the first 10–15 min of exchange data. Data were corrected for residual light scatter contributions by subtracting residual fluorescence anisotropy (r) (related to fluorescence polarization (P) as r = 2P/(3 – P)) of both donor and acceptor membranes from all experimental data. For each combination of donor and acceptor membranes, the conversion of initial rates of polarization change to initial rates of molecular sterol transfer was performed employing the respective standard curves as described in the “Appendix.”

RESULTS

Intermembrane Sterol Exchange Plasma Membranes as Donors—Plasma membrane (PM) donor DHE fluorescence polarization in the absence of acceptor membranes was stable during the time of assay (Fig. 1A, curve 1). In the presence of a 10-fold excess of PM acceptor, the polarization increased linearly for the first 10–15 min (Fig. 1A, curve 2). Conversion of initial rates of polarization change to initial rates of molecular sterol transfer yielded the initial rate of sterol transfer, 0.65 ± 0.04 pmol × min⁻¹ (Table 1). SCP-2 and L-FABP increased the initial rate of sterol transfer 6.4- and 1.5-fold, respectively (Table 1).

Replacing the plasma membrane acceptor with microsomes or mitochondria (Fig. 2A and Table 1) decreased the initial rate of spontaneous sterol transfer up to 4.3-fold, resulting in the following order: PM-PM > PM-MICRO > PM-MITO (Table 1). This striking result indicated that, although L-cell PM and MITO differ nearly 3-fold in the sterol/phospholipid ratio, the spontaneous rate of sterol transfer from sterol-rich PM to sterol-poor MITO was actually lowest, opposite to what is expected based only on the direction of the sterol concentration gradient.

Although SCP-2 stimulated all of the sterol transfer rates between the different donor-acceptor pairs, the effect was largest (27-fold increase) for sterol exchange from PM to MICRO, while PM-PM and PM-MITO sterol transfer rates were stimulated 6- and 12-fold, respectively (Table 1). The presence of SCP-2 altered the order of initial rates of sterol transfer to PM-MITO > PM-PM > PM-MITO. In contrast, L-FABP did not affect the PM-MICRO sterol transfer rate (Table 1), but caused a significant, 3-fold stimulation of sterol transfer between plasma membranes and mitochondria (PM-PM and PM-MITO).

Microsomal Membranes as Donors—Unlike PM donor membranes, the DHE fluorescence polarization signal in microsomes increased slowly and monotonically with time even in the absence of acceptor MICRO (Fig. 1B, curve 1). This observation reflected a slow transition toward an equilibrium state among heterogeneous microsomal donor membrane subfractions. Addition of a 10-fold excess of acceptor MICRO vesicles to donor MICRO shifted the kinetic equilibrium of this sterol transfer reaction from sterol exchange within the microsomal...
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was made by subtracting the fluorescence anisotropy signal of the donor alone at each corresponding time point from all experimental data points. The resultant corrected curve for the microsomal donor-microsomal acceptor membrane (MICRO-MICRO) exchange is shown in Fig. 1B (curve 2).

The initial rate of sterol exchange between microsomal donor and microsomal acceptor membranes (Table II) was 1.4-fold slower than in PM-PM exchange (Table II). There was no statistical difference between MICRO-MICRO and MICRO-MITO sterol exchange rates (Table II). The presence of SCP-2 (1.5 μM) during the exchange increased the initial rate of MICRO-MICRO sterol transfer by 3.1-fold. In contrast, L-FABP (1.5 μM) was without significant effect on the initial rate of sterol transfer between microsomal donor and microsomal acceptor membranes.

Among exchanges where microsomal membranes served as donors, replacement of acceptor MICRO with acceptor PM or MITO (Fig. 2B and Table II) dramatically decreased the spontaneous sterol transfer rates 3- and 4-fold, respectively. The combination MICRO-MITO showed the lowest initial rate of sterol transfer, while the pair MICRO-MICRO had the highest rate. The sterol molecular transfer from MICRO to PM (Table II) gave a rate 3-fold slower than from PM to MICRO (Table I). This is consistent with the direction of the cholesterol gradient between these membrane fractions. In contrast, spontaneous sterol transfer from MICRO to cholesterol-poor MITO (Table II) was dramatically decreased 13-fold as compared with MITO-MICRO (Table III), opposite to what was expected on the basis of the higher sterol/phospholipid ratio in MICRO as compared with MITO inner membrane.

Both SCP-2 and L-FABP increased the sterol transfer rate between microsomal and mitochondria membranes (MICRO-MITO) by ~3-fold. SCP-2 also stimulated MICRO-PM (~5-fold) and MICRO-MICRO (~2-fold) sterol exchanges (Table II). L-FABP had no significant effect on sterol transfer in either MICRO-PM or MICRO-MICRO donor-acceptor combinations.

Finally, SCP-2 stimulated MICRO-PM sterol transfer (Table II) much less than in the opposite direction, PM-MICRO (Table I), 5-versus 26-fold, respectively. This is consistent with SCP-2 preferentially targeting sterol transfer from PM to MICRO as opposed to the reverse direction.

Mitochondrial Membranes as Donors—Mitochondrial donor DHE polarization was stable during the time of assay in the absence of acceptor membranes (Fig. 1C, curve 1). Addition of a 10-fold excess of acceptor MITO resulted in a rapid linear increase in polarization (Fig. 1C, curve 1). Although L-cell mitochondria have a 3-fold lower cholesterol/phospholipid ra-

### Table I

| Donor-acceptor          | Initial rate of sterol transfer | SCP-2 | L-FABP |
|-------------------------|---------------------------------|-------|--------|
| PM-PM                   | 0.65 ± 0.04                     | 4.14 ± 0.64 | 0.97 ± 0.13 |
| PM-MICRO                | 0.48 ± 0.06                     | 12.8 ± 0.80 | 0.56 ± 0.09 |
| PM-MITO                 | 0.15 ± 0.03                     | 1.75 ± 0.27 | 0.44 ± 0.07 |

a p < 0.01 compared with no protein.

b p < 0.05 compared with PM-PM.

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**Fig. 1. Sterol exchange between biological membranes.** A, sterol exchange between plasma membranes. Data represent the DHE fluorescence polarization as a function of time in the donor vesicles before (7 μg of protein/ml; curve 1) and after addition of acceptor plasma membranes (70 μg of protein/ml) to the donor vesicles (7 μg of protein/ml) at time 0 (curve 2). The straight line is the linear fit of the first 10 min of exchange (r² = 0.90). B, sterol exchange between microsomes. Data are the kinetics of DHE fluorescence polarization in microsomal donor membranes before (7 μg of protein/ml; curve 1) and immediately after addition of acceptor membranes (70 μg of protein/ml) to a donor vesicle suspension (7 μg of protein/ml; curve 3). Experimental data were corrected for the instability of the donor, yielding the resultant curve 2 (see text for more details on the correction procedure). The straight line shows the linear fit of the first 10 min of exchange (r² = 0.9). C, sterol exchange between mitochondria. Data are the kinetics of DHE fluorescence polarization in the solution of donor membranes (7 μg of protein/ml; curve 1) and upon addition of acceptor membranes (70 μg of protein/ml) to the donor vesicles (7 μg of protein/ml; curve 2). The first 10 min of sterol exchange (curve 2) is best described by the linear dependence (r² = 0.9) as it is demonstrated by the straight line.

**TABLE I**

Spontaneous and protein-mediated sterol exchange between plasma membrane donors and different acceptors

The initial rates of sterol transfer within different donor-acceptor pairs were calculated from the observed DHE fluorescence polarization change by using the following standard curves (for more details, see the “Appendix”): PM-PM: P = b₀ + b₁·X₀ + b₂·X₂, b₀ = 0.3155, and b₁ = −0.131; PM-MICRO: P = b₀ + b₁·X₀ + b₂·X₂ + b₃·X₃, b₀ = 0.2569, b₁ = −0.086, b₂ = −0.1685, and b₃ = 0.0396; and PM-MITO: P = b₀ + b₁·X₀ + b₂·X₂ + b₃·X₃ + b₄·X₄, b₀ = 0.3164, b₁ = 0.0082, b₂ = −0.2289, and b₄ = 0.0612. SCP-2 or L-FABP was 1.5 μM. Values represent the mean ± S.E. (n = 3–11).

| Donor-acceptor          | Initial rate of sterol transfer | SCP-2 | L-FABP |
|-------------------------|---------------------------------|-------|--------|
| PM-PM                   | 0.65 ± 0.04                     | 4.14 ± 0.64 | 0.97 ± 0.13 |
| PM-MICRO                | 0.48 ± 0.06                     | 12.8 ± 0.80 | 0.56 ± 0.09 |
| PM-MITO                 | 0.15 ± 0.03                     | 1.75 ± 0.27 | 0.44 ± 0.07 |

a p < 0.01 compared with no protein.

b p < 0.05 compared with PM-PM.

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**FIG. 2. Sterol exchange between microsomes and mitochondria.** A, the initial rate of sterol exchange between microsomes (70 μg of protein/ml) and mitochondria (70 μg of protein/ml) in the presence of SCP-2 (1.5 μM) and L-FABP (1.5 μM) was used to calculate the kinetic parameters of sterol exchange. The linear fit (r² = 0.9) of the first 10 min of exchange is shown (solid line). B, the initial rate of sterol exchange between microsomes and mitochondria in the presence of SCP-2 (1.5 μM) and L-FABP (1.5 μM) was used to calculate the kinetic parameters of sterol exchange. The linear fit (r² = 0.9) of the first 10 min of exchange is shown (solid line). C, the initial rate of sterol exchange between microsomes and mitochondria in the presence of SCP-2 (1.5 μM) and L-FABP (1.5 μM) was used to calculate the kinetic parameters of sterol exchange. The linear fit (r² = 0.9) of the first 10 min of exchange is shown (solid line).
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**Fig. 2.** Sterol exchange between dissimilar donor and acceptor membranes. A, sterol exchange between plasma membranes and microsomes. Data represent the DHE fluorescence polarization as a function of time in the plasma membrane donor vesicles alone (7 μg of protein/ml; curve 1) and after addition of microsomal acceptor membranes (70 μg of protein/ml) to the plasma membrane donor vesicles (7 μg of protein/ml) at time 0 (curve 2). The straight line is the linear fit of the first 10 min of exchange (r² = 0.90). B, sterol exchange between microsomes and plasma membranes. Data are the kinetics of DHE fluorescence polarization in microsomal donor membranes alone (7 μg of protein/ml; curve 1) and immediately after addition of acceptor plasma membranes (70 μg of protein/ml) to a microsomal donor vesicle suspension (7 μg of protein/ml; curve 2). Experimental data were corrected for the instability of the donor, yielding the resultant curve 2 (see text for more details on the correction procedure). The straight line shows the linear fit of the first 10 min of exchange (r² = 0.9). C, sterol exchange between mitochondria and microsomes. Data are the kinetics of DHE fluorescence polarization in mitochondrial donor membranes (7 μg of protein/ml; curve 1) and upon addition of microsomal acceptor membranes (70 μg of protein/ml) to the mitochondrial donor vesicles (7 μg of protein/ml; curve 2). The first 10 min of sterol exchange (curve 2) is best described by the linear dependence (r² = 0.9) as it is demonstrated by the straight line.

The initial rates of sterol transfer within different donor-acceptor pairs were calculated from the observed DHE fluorescence polarization change by using the following standard curves (for more details, see the "Appendix"): MITO-MITO: P = b₀ + b₁ · X₁ + b₂ · X₂, b₀ = 0.2685, b₁ = 0.0042, and b₂ = −0.0303; MICRO-PM: P = b₀ + b₁ · X₁ + b₂ · X₂, b₀ = 0.3331, b₁ = −0.057, and b₂ = −0.0344; and MICRO-MITO: P = b₀ + b₁ · X₁ + b₂ · X₂, b₀ = 0.3359, b₁ = −0.062, and b₂ = −0.044. SCP-2 or L-FABP was 1.5 μM. Values represent the mean ± S.E. (n = 3–8).

| Donor-acceptor | Initial rate of sterol transfer |
|----------------|--------------------------------|
|                | pmol × min⁻¹                  |
| No protein     | 0.42 ± 0.06                   |
| SCP-2          | 0.92 ± 0.18 *                 |
| L-FABP         | 0.28 ± 0.09                   |

* p < 0.05 compared with no protein.

**Table III**

Spontaneous and protein-mediated sterol exchange between mitochondrial donors and different acceptors

The initial rates of sterol transfer within different donor-acceptor pairs were calculated from the observed DHE fluorescence polarization change by using the following standard curves (for more details, see the "Appendix"): MITO-MITO: P = b₀ + b₁ · X₁ + b₂ · X₂, b₀ = 0.3309, b₁ = 0.0069, and b₂ = −0.075; MICRO-PM: P = b₀ + b₁ · X₁ + b₂ · X₂, b₀ = 0.3142, b₁ = 0.0235, and b₂ = −0.1129; and MICRO-MITO: P = b₀ + b₁ · X₁ + b₂ · X₂, b₀ = 0.2538, b₁ = −0.0986, b₂ = −0.1433, and b₃ = 0.0284. SCP-2 or L-FABP was 1.5 μM. Values represent the mean ± S.E. (n = 3–8).

| Donor-acceptor | Initial rate of sterol transfer |
|----------------|--------------------------------|
|                | pmol × min⁻¹                  |
| No protein     | 0.62 ± 0.13                   |
| SCP-2          | 1.4 ± 0.08 *                  |
| L-FABP         | 0.52 ± 0.05                   |

* p < 0.05 compared with no protein.

**DISCUSSION**

Despite the substantial advances made in our understanding of cholesterol transport in the vasculature, relatively little is known regarding intracellular cholesterol trafficking pathways (4, 43). One potential complication in studying cholesterol trafficking in intact cells is that multiple types of donor and accep-
Thus, the data do not support the commonly held assumption that the rate-limiting step in the process of sterol exchange between biomembranes is necessarily the rate of sterol desorption from a donor membrane. Therefore, in addition to the desorption rate, the nature of the acceptor membrane can have a significant role in this process.

Another important observation made from this study is that SCP-2 is much more effective and has different specificity than L-FABP in enhancing the initial rates of intramembrane sterol trafficking in vitro. The concentration of SCP-2 used in this study (1.5 μM) falls in the range of 1–50 μM that is present in normal tissues (47, 48). This concentration was highly effective in stimulating sterol transfer in both similar and dissimilar donor-acceptor pairs (Figs. 1–3 and Tables I–III). The largest stimulatory effect of SCP-2 was seen in sterol transfer from plasma membranes to microsomes, where the rate was increased ~27-fold, and from plasma membranes to mitochondria (~12-fold). The lowest rate enhancement by SCP-2 was observed in homogeneous sterol exchange between microsomes (~2-fold) and mitochondria (~2-fold). More important, SCP-2 enhanced sterol trafficking from PM to MICRO and from PM to MITO to a much greater extent than in the reverse directions (27-fold versus 5-fold and 12-fold versus 4-fold, respectively).

Thus, SCP-2 stimulates sterol transfer down a concentration gradient from cholesterol-rich PM to relatively cholesterol-poor MICRO and MITO. L-FABP under the same conditions caused a significant increase in the initial sterol transfer rate only in a few donor-acceptor pairs: PM-PM (~1.5-fold), PM-MITO (~3-fold), and MICRO-MITO (~3-fold). Although it appears that L-FABP was much less effective in stimulating intermembrane sterol transfer, it must be recognized that the cytosolic L-FABP concentration in liver and intestine is up to 64-fold higher than that of SCP-2 (48, 49). Furthermore, subcellular compartmentalization of SCP-2 differs markedly from that of L-FABP. SCP-2 is present not only in the cytosol, but also in peroxisomes and/or mitochondria (50). In contrast, L-FABP is primarily cytosolic, with some enrichment near the endoplasmic reticulum (49, 51, 52).

It should be noted, that in comparison with L-FABP, the role of SCP-2 in sterol transfer has been investigated more extensively. To our knowledge, there are only a few examples (4, 21, 29) where an effect of L-FABP on intermembrane sterol transfer was tested. It appears that L-FABP enhances sterol exchange between plasma membranes (4), but has no influence on sterol transfer between model lipid vesicles (29). It was also shown that spontaneous sterol exchange between plasma membranes obtained from cells transfected with cDNA coding for L-FABP is significantly faster in comparison with nontransfected cell lines (21). These observations serve as indirect evidence that expression of L-FABP alters the transferability of sterol between biological membranes and thereby alters the intracellular sterol distribution. Similar studies with transfected cells overexpressing SCP-2 remain to be performed.

The mechanism by which cytosolic lipid carrier proteins stimulate sterol transfer between biological membranes is not fully understood. A preponderance of data indicate that SCP-2 or L-FABP binds to the membrane surface and then interacts with sterol in the membrane, thereby stimulating sterol desorption from the membrane (46, 53–55). Under certain circumstances, differences in the surface charge between donor or acceptor model membranes can modulate the extent and direction of SCP-2-mediated sterol transfer (46).

The data presented in this paper do not allow assignment of a unique role to SCP-2 in each of the membrane combinations tested in vitro for intracellular protein-mediated sterol trafficking. However, the new observations made may be significant to sterol metabolism as follows.
Sterol movement within microsomes, a membrane fraction accounting for the majority of sterol synthesis and cholesterol esterification, was observed to be rapid. However, sterol synthesis does not occur in a single microsomal fraction, but a product of one intermediate reaction must be transferred to the next enzymatic step in another microsomal subfraction to accomplish complete cholesterol synthesis (56, 57). It may be postulated that this fast intramicrosomal sterol trafficking may play an important role in sterol biosynthesis. This also suggests that the process of sterol transfer in this microsomal sterol synthetic machinery is mediated by a cytosolic protein. SCP-2 is an appropriate candidate protein that may mediate this sterol transfer.

SCP-2-mediated MICRO-PM and MITO-PM pathways may play an important role in reverse cholesterol transport, i.e. removing free cholesterol from nonspecialized cells, which do not produce steroid hormones or bile acids. The data show that SCP-2 enhanced MICRO-PM sterol transfer by 5-fold in vitro. Recent results with intact human fibroblasts transfected with SCP-2 antisense oligonucleotides showed a 5-fold reduction in rapid cholesterol transfer from the endoplasmic reticulum to plasma membranes (58). This observation is consistent with the present findings with isolated membrane fractions in vitro. The SCP-2-mediated MICRO-MITO and PM-MITO pathways may be involved in supplying cholesterol to mitochondria for oxidation to steroid hormones in steroidogenic cells (59, 60).

SCP-2-mediated cholesterol transfer from PM to MICRO may be an important rapid step in the lysosomal → PM → microsomal sterol trafficking pathway in which low density lipoprotein-derived cholesterol either becomes re-esterified by microsomal acyl-CoA:cholesterol O-acyltransferase or is microsomally oxidized to hydroxymethylglutaryl-CoA reductase-regulatory sterols. SCP-2 is a potent stimulator of microsomal acyl-CoA:cholesterol O-acyltransferase in vitro (42, 61, 62). However, the relevance of SCP-2 to cholesterol esterification in vivo is unclear. Intracellular esterification of plasma membrane-derived cholesterol is enhanced in transfected L-cell fibroblasts expressing SCP-2. In contrast, published reports utilizing mutant Chinese hamster ovary (63) and hepatoma (64) cell lines provide some doubt as to whether SCP-2 functions in enhancing cholesterol esterification in vivo. Because it appears unlikely that the genetic defect in the mutant Chinese hamster ovary cells or hepatoma cells is in the SCP-2 gene, alterations in SCP-2 expression in the mutant and hepatoma cells may represent secondary effects. In addition, Puglielli et al. (58) have shown that both a vesicular and an SCP-2-mediated sterol transfer pathway are operative in human fibroblasts. Thus, reductions in SCP-2 expression (63, 64) may be compensated by increased vesicular cholesterol transport (58). Consequently, it is difficult to make clear-cut interpretations from correlations between SCP-2 expression and cholesterol esterification in the mutant cell lines.

APPENDIX

Standard Curves for the Exchange Assay—The standard curve analysis originally derived for model membranes by Butko et al. (41) was refined and applied to L-cell plasma membranes as described (22). Separate standard curves were also constructed for L-cell microsomal and mitochondrial membranes as well as for dissimilar donor-acceptor pairs involving plasma membranes, microsomes, and mitochondrial membranes.

2 E. J. Murphy and F. Schroeder, unpublished data.
DHE fluorescence polarization and DHE mass were measured in membranes isolated from L-cells grown in the presence of varying concentrations of fluorescent sterol (Fig. 4). DHE fluorescence polarization in microsomal (Fig. 4A) and mitochondrial (Fig. 4B) membranes decreased with increasing DHE content (as determined by HPLC), in agreement with the earlier model membrane (41) and plasma membrane (22) data. The data were computer-analyzed and best fit to Equation 1 with the parameters $P_0 = 0.2716$, $B = 0.0597$, and $r^2 = 0.87$ (microsomes) and $P_0 = 0.3397$, $B = 0.0358$, and $r^2 = 0.90$ (mitochondria):

$$P = P_0 C (B + C)$$  \hspace{1cm} (Eq. 1)

where $P$ is the measured fluorescence polarization and $C$ is the concentration of DHE in membranes. $P_0$ represents the DHE fluorescence polarization at its infinite dilution in membranes. The calculated values for plasma membranes were $P_0 = 0.3366$ and $B = 0.0576$ (22).
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Equation 1 describes the polarization dependence in solutions containing only donor membranes. To construct a standard curve for the actual assay, contributions from the acceptor vesicles must also be determined. The relative concentrations of DHE present in the donor ($X_d$) and acceptor ($X_a$) vesicles can be described by Equations 2 and 3:

$$X_d = C_d/C_i$$  \hspace{1cm} (Eq. 2)

$$X_a = 1 - X_d = 1 - (C_d/C_i)$$  \hspace{1cm} (Eq. 3)

where $C_i$ and $C_d$ are the initial and current DHE concentrations in the donor membrane, respectively. The anisotropy of any mixture of donor and acceptor can then be expressed as in Equation 4:

$$r = r_0 + (X_d + X_a - D) + (1 - X_d)(1 + 10(1 - X_d)/D)$$  \hspace{1cm} (Eq. 4)

where $r$ is the measured fluorescence anisotropy and $r_0$ is anisotropy at infinite dilution of DHE in membranes. The value of $r_0$ can be easily calculated from $P_0$ (Equation 5).

$$r_0 = -2 \cdot P_0 / 3 - P_0$$  \hspace{1cm} (Eq. 5)

The constant D in Equation 4 is related to the constant B in Equation 1 as follows (Equation 6):

$$D = Z \cdot B \cdot (1 + (r_0/2))$$  \hspace{1cm} (Eq. 6)

where $Z$ is the mole percent of DHE in the total membrane lipid. Parameter $Z$, in turn, can be estimated on the basis of Equation 7:

$$Z = (P_0 - P)/P \cdot B$$  \hspace{1cm} (Eq. 7)

where $P$ is the DHE fluorescence polarization in the donor membranes in the absence of an acceptor and $P_0$ and $B$ are the parameters from Equation 1. The first term in Equation 4 describes the DHE fluorescence anisotropy in the donor vesicles, while the second term represents the acceptor fraction for the total fluorescence anisotropy in the particular donor-acceptor combination. Hence, it should be clear that standard curves for dissimilar donor-acceptor pairs can be designed on the basis of Equation 4 with the correspondent combination of D values in its two terms.

The value of fluorescence anisotropy was calculated for the range of $X_d$ from 0 to 1 and then converted to polarization using Equation 8.

$$P = 3 \cdot r/(2 + r)$$  \hspace{1cm} (Eq. 8)

The generated standard curves for similar and dissimilar donor-acceptor pairs are presented in Fig. 5 (A–C) and Fig. 6 (A–C), respectively. Then, the calculated fluorescence polarization values for all possible donor-acceptor combinations were computer-fit to polynomial equations. The corresponding data are summarized in Tables I and II, with the number of terms in the polynomial equation required to give a good fit varying from two to four depending on donor and acceptor type.

Initial Rate of Sterol Transfer—Shown for plasma membranes (Fig. 5A), microsomes (Fig. 5B), and mitochondria (Fig. 5C) are the calculated standard curves for similar donor and acceptor pairs. The corresponding experimental data reflecting an increase in fluorescence polarization as a function of time after addition of acceptor membranes are shown in Fig. 1 (A–C). For each donor-acceptor standard curve, the polarization increased linearly when the remaining fraction of DHE in the donor membranes ($X_{d0}$) was from 0.8 to 1.0 (Fig. 5, A–C). This observation predicts that in an exchange assay, the initial (up to 20%) DHE transfer should be directly proportional to the change of fluorescence polarization. This prediction was supported by the experimental data shown in Fig. 1 (A–C), where the straight lines represent the computer best linear fit of experimental points during the first 10–20 min of sterol exchange.

The precise equation that links the initial fluorescence polarization rate to the initial rate of molecular DHE transfer between biological membranes was derived from the respective standard curves. For example, exchange between mitochondria was described by the following standard curve (Equation 9).

$$P = b_0 + b_1 \cdot X_d + b_2 \cdot X_d^2$$  \hspace{1cm} (Eq. 9)

Taking the derivative for both parts of Equation 9, one obtains the following (Equation 10).

$$(dP/dt) = b_1 \cdot (dX_d/dt) + 2b_2 \cdot X_d \cdot (dX_d/dt)$$  \hspace{1cm} (Eq. 10)

When $t \to 0$, $X_d \to 1$ (Fig. 4C), and Equation 10 can be rearranged in the following manner (Equation 11).

$$1/(b_1 + 2b_2) \cdot (dP/dt)_{t=0} = (dX_d/dt)_{t=0}$$  \hspace{1cm} (Eq. 11)

Taking into account the donor membrane concentration (7 µg of protein/ml), the mitochondrial sterol concentration (530 pmol/µg of protein), the initial concentration of DHE in the donor membranes (8 mol %), and the numerical values of the parameters $b_1$ and $b_2$ (0.0069 and $-0.075$, respectively), Equation 11 can be rewritten in its final form (Equation 12).

$$(d(dH/E/dt))_{t=0} = -2.1 \text{nmol} \cdot (dP/dt)_{t=0}$$  \hspace{1cm} (Eq. 12)

Substitution of the initial rate of fluorescence polarization change $(dP/dt)_{t=0}$ by its respective measured value (3.0 ± 0.6 units × 10^-4 min^-1) (see Fig. 1C) yielded the initial rate of molecular sterol transfer (0.62 ± 0.13 pmol × min^-1). The same algorithm was applied to all donor and acceptor combinations studied, and the calculated values of molecular sterol transfer rate were collected in Tables I–III.

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