Differential Mobilization of Newly Synthesized Cholesterol and Biosynthetic Sterol Precursors from Cells*

Sari Lusa, Sanna Heino, and Elina Ikonen‡
From the Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland

Previous work demonstrates that the biosynthetic precursor of cholesterol, desmosterol, is released from cells and that its efflux to high density lipoprotein or phosphatidylcholine vesicles is greater than that of newly synthesized cholesterol (Johnson, W. J., Fischer, R. T., Phillips, M. C., and Rothblat, G. H. (1995) J. Biol. Chem. 270, 25037–25046). Here we report that the release of individual precursor sterols varies with the efflux of newly synthesized zymosterol being greater than that of lathosterol and both exceeding that of newly synthesized cholesterol when using either methyl-β-cyclodextrin or complete serum as acceptors. The transfer of newly synthesized lathosterol to methyl-β-cyclodextrin was inhibited by actin polymerization but not by Golgi disassembly whereas that of newly synthesized cholesteryl was inhibited by both conditions. Newly synthesized lathosterol associated with cellular detergent-resistant membranes more rapidly than newly synthesized cholesteryl. Upon efflux to serum, newly synthesized cholesterol precursors associated with both high and low density lipoproteins. Stimulation of the formation of direct endoplasmic reticulum-plasma membrane contacts was accompanied by enhanced efflux of newly synthesized lathosterol but not of newly synthesized cholesterol to serum acceptors. The data indicate that the efflux of cholesterol precursors differs not only from that of cholesterol but also from each other, with the more polar zymosterol being more avidly effluxed. Moreover, the results suggest that the intracellular routing of cholesterol precursors differs from that of newly synthesized cholesterol and implicates a potential role for the actin cytoskeleton and endoplasmic reticulum-plasma membrane contacts in the efflux of lathosterol.

Virtually all the organs of the body synthesize cholesterol, with extrahepatic tissues accounting for a significant fraction of whole body sterol production (1). Cholesterol is synthesized from acetyl-CoA via the mevalonate pathway that initially produces farnesyl diphosphate, a precursor for squalene, dolichol, heme α, ubiquinone, and isoprenylated proteins. The committed step in cholesterol synthesis is the cyclization of squalene to lanosterol. From this compound, cholesterol is synthesized in a 19-step process involving the activity of nine different enzymes (2). Recent data indicate that sterols regulate the pathway both at the early (i.e. via hydroxymethylglutaryl-coenzyme A reductase) and postlanosterol steps (3). The late steps of cholesterol synthesis can proceed via lathosterol and 7-dehydrocholesterol or via desmosterol to cholesterol. Interestingly, the relative importance of the two pathways may shift in vivo, e.g. during aging (4).

Cholesterol biosynthesis is critically important for human development and cannot be compensated for by increasing the uptake of cholesterol from exogenous sources. This is exemplified by an increasing number of inborn errors of metabolism that are attributed to mutations in cholesterol biosynthetic enzymes (5). The prototype of these disorders, Smith-Lemli-Opitz syndrome (SLOS) is caused by deficiency of 7-dehydrocholesterol reductase, the last step in cholesterol synthesis via the lathosterol pathway. More recently, other multiple malformation/mental retardation syndromes, including lathosterolemia, have been characterized (6). In these patients, cholesterol precursors may constitute up to ~10% of total cellular and plasma sterols. Importantly, cholesterol precursors are also found in normal human plasma, at concentrations roughly 1:1000 of that of cholesterol (7, 8). The plasma levels of lathosterol and desmosterol are commonly used as measures of the cholesterol biosynthetic activity of the individual. Recent data suggest that these values are highly heritable (9, 10) and could potentially be used to predict individual responsiveness to the cholesterol-lowering regimen (11, 12).

Cholesterol biosynthetic enzymes are localized in the cytosol as well as rough and smooth endoplasmic reticulum (ER), both the rate-limiting and the last enzyme of the pathway (hydroxymethylglutaryl-CoA reductase and 7-dehydrocholesterol reductase, respectively) being integral membrane proteins of the ER (13–15). Several steps of the pathway also occur in peroxisomes. However, the absence of functional peroxisomes does not lead to deficiency of cholesterol biosynthetic enzymes (16). The transfer of cholesterol from its site of synthesis in the ER to the plasma membrane and extracellular acceptors has been investigated in a number of studies (for reviews see Refs. 17 and 18). Instead, the transfer of sterol precursors has so far received little attention despite the pioneering observations by Lange et al. (19) and Johnson and co-workers (20, 21) that indicate clear differences in the behavior of cholesterol and its biosynthetic precursors.

Lange and co-workers (22, 23) reported that in fibroblasts at least three cholesterol precursors, lanosterol, zymosterol, and 7-dehydrocholesterol were highly concentrated in the plasma...
membrane. Moreover, newly synthesized zymosterol was found to move to the plasma membrane faster than cholesterol, with a half-time of 9 min (that of cholesterol being 18 min). In contrast, in Mca-RH7777 cells the rate of transport of newly synthesized desmosterol was found to be roughly equal to that of cholesterol, with a half-time of ~30 min for cholesterol and ~40 min for desmosterol (24). Lange et al. (19) further reported that plasma membrane zymosterol turned over rapidly by internalization and became converted to cholesterol. On the other hand, Johnson et al. (20) showed that sterol precursors were not only enriched in the plasma membrane but were rapidly effluxed from cells to high density lipoprotein and phosphatidylcholine vesicles. The major biosynthetic sterol released from Chinese hamster ovary cells was reported to be desmosterol or desmosterol/cholesterol. Moreover, we provide evidence suggesting that the faster efflux of zymosterol is coupled to a cellular circuit different from that of newly synthesized cholesterol and that its release to physiological acceptors can be modulated differently from that of newly synthesized cholesterol.

EXPERIMENTAL PROCEDURES

Materials—Media and reagents for cell culture were from Invitrogen. Lipoprotein-deficient serum (LPDS) was prepared as in Ref. 43. [4-14C]Cholesterol (specific activity, 55.0 mCi/mmol), [13H]acetate (specific activity, 10.0 Ci/mmol), Redivue Pro [35S]Met/Cys labeling mixture (specific activity, 1,000 Ci/mmol), protein A-Sepharose, and Amplify Fluorographic Reagent were from Amersham Biosciences. Brefeldin A (BFA) was from Epicentre Technologies and lovastatin from Merck Sharp & Dohme. Jasplakinolide was kindly provided by Prof. Philip Crews (Dept. of Chemistry and Biochemistry, Univ. of California, Santa Cruz). Cycloheximide, protease inhibitors, blue dyed latex mixture (specific activity, 1,000 Ci/mmol), protein A-Sepharose, and Amplify Fluorographic Reagent were from Amersham Biosciences. Brefeldin A (BFA) was from Epicentre Technologies and lovastatin from Merck Sharp & Dohme. 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tracted lipids by TLC. The cholesterol TLC spot was then analyzed by silver ion HPLC and the fraction of $[^{3}H]$cholesterol plotted. As shown in Fig. 1a, the fraction of $[^{3}H]$cholesterol increased with increasing chase time but most of the $[^{3}H]$radioactivity in the TLC cholesterol spot was actually not cholesterol. The result is in line with that reported by Johnson et al. (20) using CHO cells and a longer $[^{3}H]$acetate labeling time.

Prolonged incubation at 14°C during $[^{3}H]$acetate labeling has been used to accumulate newly synthesized cholesterol intracellularly prior to chasing at 37°C (26, 31). Therefore, the fraction of cholesterol present in the TLC spot was also monitored under these conditions. At chase times under 30 min when cholesterol was postulated to undergo rapid movement, less than 50% of the TLC spot represented $[^{3}H]$cholesterol (Fig. 1b). According to HPLC analysis, $[^{3}H]$lathosterol was one of the major $[^{3}H]$acetate-derived products co-migrating in the TLC spot, representing 35% of the dpms analyzed at 0–10 min and 25% at 30 min of chase (data not shown). The result suggests that the use of TLC alone could yield misleading results and reinforces the necessity of using methods with high resolving ability for accurate separation of cellular sterols.

Next, the levels of $[^{3}H]$acetate-derived newly synthesized cholesterol and its biosynthetic precursor sterols were measured by HPLC from fibroblastic (BHK and NIH3T3) and hepatic (HuH7) cell lines. Prior to $[^{3}H]$acetate labeling, the cells were sterol-starved by culturing for 2 days in lipoprotein-deficient medium. The cells were pulse-labeled with $[^{3}H]$acetate for 15 min and chased for 30 or 60 min. Both the rate and efficiency of $[^{3}H]$acetate incorporation into cholesterol varied considerably between similarly cultured cells (Fig. 2). HuH7 and NIH3T3 cells produced $[^{3}H]$cholesterol more efficiently than BHK cells that were slower in synthesizing cholesterol and contained larger fractions of the precursor sterols.

BHK cells, $[^{3}H]$zymosterol represented the major sterol peak by HPLC analysis both at 30 and 60 min of chase (Fig. 2, a and b). BHK cells also contained significant levels of both $[^{3}H]$lathosterol and $[^{3}H]$desmosterol, whereas in HuH7 and NIH3T3 cells, lathosterol represented the major precursor sterol, and only minor amounts of desmosterol were detected (Fig. 2b). The results in HuH7 cells are in line with those observed in another...
hepatic cell line, HepG2, with cholesterol as the main biosynthetic sterol product (20). On the other hand, some fibroblastic cells synthesize cholesterol efficiently while others do not, as exemplified by NIH3T3 and BHK cells, respectively.

**Efflux of Cholesterol and Its Biosynthetic Precursors to Methyl-β-cyclodextrin**—To examine the efflux of newly synthesized sterols, the release of [3H]acetate-derived sterols to methyl-β-cyclodextrin was analyzed from BHK and HuH7 cells after increasing chase times. For HuH7 cells, the [3H]acetate labeling was shortened to 5 min to increase the proportion of radio-labeled precursor sterols. Methyl-β-cyclodextrin was added to the cells for 5 min at 37 °C either immediately after the labeling or after increasing chase times in serum-free medium (in the presence of statin and unlabeled mevalonate). The cyclodextrin concentration was titrated such that the efflux of pre-labeled cellular [14C]cholesterol was ~25%. The efflux of [3H]cholesterol increased gradually with increasing chase times in both cell lines, being somewhat faster in HuH7 than in BHK cells as shown previously (27) (Fig. 3a). In both cell lines, the efflux of newly synthesized [3H]lathosterol was greater than that of newly synthesized [3H]cholesterol at all time points analyzed (Fig. 3a). In BHK cells, the major precursor [3H]zymosterol was very efficiently recovered by cyclodextrin and was virtually absent from the cells, yielding a very high efflux percentage (82.4 ± 0.3% at 5 min, 80.5 ± 1.0% at 60 min, and 52.8 ± 2.39% at 120 min of chase). Accordingly, when the dpm of [3H]labeled sterols in the medium were plotted in BHK cells, the overwhelming majority represented zymosterol at short chase times (5–60 min) (Fig. 3b). By contrast, [3H]desmosterol was least readily effluxed of the BHK cell sterol precursors, the [3H]desmosterol efflux percentage being intermediate between [3H]cholesterol and [3H]lathosterol (Fig. 3a and b). The more efficient completion of cholesterol biosynthesis in HuH7 cells was paralleled by the release of fewer precursors and correspondingly more of newly synthesized cholesterol to the acceptor (Fig. 3b).

We have previously shown that the efflux of newly synthesized cholesterol to cyclodextrin is moderately inhibited in both BHK and HuH7 cells by BFA (27). However, we now observed that BFA had no effect on the cyclodextrin availability of newly synthesized lathosterol under the same conditions (Fig. 4a and b). In search of additional modulators of newly synthesized sterol efflux we tested the effect of a membrane-permeant promoter of actin polymerization, the marine sponge toxin, jasplakinolide. We found that this compound inhibited slightly but reproducibly the efflux of both newly synthesized cholesterol and lathosterol (Fig. 4a and b). Interestingly, for newly synthesized cholesterol the effect was apparently additive with that of BFA, suggesting that jasplakinolide affected a Golgi-bypass route of cholesterol transport (Fig. 4a). This was also in line with the observation that the jasplakinolide treatment had no effect on albumin secretion from the cells (Fig. 4c). The combination of BFA and jasplakinolide was not significantly

![Fig. 3. Efflux of biosynthetic sterols to methyl-β-cyclodextrin.](image)

**Mobilization of Cholesterol Precursors from Cells**

![Fig. 4. Effect of jasplakinolide on newly synthesized sterol and protein transport.](image)
more effective than jasplakinolide alone in inhibiting the efflux of lathosterol (Fig. 4b). Similar inhibition by jasplakinolide on the efflux of newly synthesized lathosterol to methyl-β-cyclodextrin was observed in BHK cells (data not shown).

Association of Newly Synthesized Lathosterol with Detergent-resistant Membranes—Next, the association of newly synthesized lathosterol and cholesterol with detergent-resistant membrane fractions (DRMs) was compared. We have earlier shown that newly synthesized cholesterol was initially found in Triton X-100 soluble membranes but upon chasing, gradually associated with DRMs, kinetically closely paralleling its availability for efflux to cyclodextrin (27). We now observed that newly synthesized lathosterol acquired detergent resistance more rapidly than newly synthesized cholesterol in the same cells, with 35–40% found in DRMs already at 5 min of chase while at that time point, only ∼20% of newly synthesized cholesterol was detergent resistant (Fig. 5). At 15 min of chase, the difference between the detergent resistance of newly synthesized lathosterol and cholesterol was still considerable (50–55% and 30–35% in DRMs, respectively; Fig. 5c) but started to level off at longer chase times (Fig. 5b). At 1–2 h of chase, the Triton X-100 solubility of newly synthesized lathosterol and cholesterol were closely similar (Fig. 5b and data not shown) suggesting that the differences in the phase behavior of the two lipids were not due solely to their structural differences. Rather, the differential, time-dependent partitioning between the detergent soluble and resistant phases could reflect differential cellular itineraries of the biosynthetic sterols.

Efflux of Cholesterol and Its Biosynthetic Precursors to Serum—Next, we analyzed the release of newly synthesized cholesterol and sterol precursors to physiological acceptors. Complete serum was used as the acceptor because efflux to serum is more efficient than to isolated particles, such as HDL or apolipoprotein A-I. In this system, HuH7 or BHK cells prelabeled for 48 h with [14C]cholesterol and thereafter pulse-labeled with [3H]acetate for 5 or 15 min, were incubated with increasing chase times in the presence of 20% serum containing lovastatin and mevalonate. Lipids from the cells and medium were extracted and analyzed by TLC and HPLC.

As expected, an increasing fraction of cellular [14C]cholesterol was released to serum with increasing chase time (Fig. 6a). The shortest efflux time at which newly synthesized sterols could reliably be detected from the efflux medium was 30 min (Fig. 6a). Notably, [14C]cholesterol was effluxed preferentially compared with [3H]cholesterol during the entire chase period. Instead, [3H]lathosterol efflux exceeded that of [14C]cholesterol at all time points analyzed in HuH7 cells and from 1 h of chase onwards in BHK cells. Interestingly, as in the case of the cyclodextrin acceptor, [3H]zymosterol was the predominant sterol released from BHK cells at short chase times and displayed very rapid efflux kinetics, with over 40% effluxed in 1 h and ∼80% in 3 h (Fig. 6a). In HuH7 cells the amount of [3H]zymosterol was negligible, and its efflux could not be reliably measured. The same was true for [3H]desmosterol in both cell types (data not shown). Evidently, the high efflux percentages for [3H]lathosterol and in particular [3H]zymosterol, reflect efficient removal of the sterol precursors from the cells. However, the proportion of newly synthesized cholesterol in the medium [3H]-labeled sterols increased steadily so that at 2–4 of chase, cholesterol was the major newly synthesized sterol effluxed from the cells (Fig. 6b).

To identify which lipoprotein fractions newly synthesized cholesterol, zymosterol, and lathosterol associated with, cells labeled with [14C]cholesterol and [3H]acetate as above were incubated with 20% serum-containing medium for 2 h. The efflux medium was then analyzed by gel filtration on a Superose 6HR column (32). The experiment was carried out in BHK cells because of the small amount of radiolabeled precursors effluxed from HuH7 cells. Moreover, lipoprotein assembly and secretion in hepatic cells might complicate the interpretation of the data. The elution profiles of the prelabeled [14C]cholesterol, [3H]-labeled sterols increased steadily so that at 2–4 of chase, cholesterol was the major newly synthesized sterol effluxed from the cells (Fig. 6b).

Fig. 5. Detergent solubility of newly synthesized lathosterol and cholesterol. HuH7 cells cultured and labeled as in the legend to Fig. 3 were chased for 5, 15, 30, or 60 min. The cells were lysed in ice-cold buffer containing 1% Triton X-100 and fractionated by Optiprep (OP) gradient centrifugation. Six fractions were collected from the top and lipids extracted and analyzed by TLC and HPLC. a, distribution of newly synthesized lathosterol and cholesterol in the gradient is shown as percentage of the sterol in each gradient fraction. b, percentage of radioactive sterols in the two top fractions representing DRMs is plotted at each chase time analyzed. In addition to newly synthesized lathosterol and cholesterol, the percentage of prelabeled cellular [14C]cholesterol recovered in DRMs from the same samples is shown. c, difference between the association of newly synthesized lathosterol and cholesterol with DRMs is statistically significant. *, [3H]lathosterol versus [3H]cholesterol in DRMs; p < 0.001.
quired by HDL and then transferred to LDL (Fig. 7e). The proportions of esterified newly released cellular sterols at 2 h of incubation were negligible (data not shown).

Promotion of ER-Plasma Membrane Contacts Is Associated with Enhanced Transfer of Newly Synthesized Lathosterol to Serum Acceptors—Considering the rapid mobilization of cholesterol precursors from cells, we speculated whether direct ER-plasma membrane contacts could facilitate this movement. It has recently been shown that ER can fuse with the plasma membrane to provide a source of membrane for the uptake of foreign material by phagocytosis (33). Within 15 min of feeding cells with inert particles (latex beads), ER chaperones such as GRP94, BiP, PDI, and calreticulin are redistributed to the phagocytic cup that forms as a specialization of the plasma membrane. Upon phagosome maturation, successive waves of ER become associated with its membrane. Although professional phagocytes, such as monocyte-macrophages and polymorphonuclear granulocytes, are most efficient in engulfing foreign particles the process occurs in a wide variety of eukaryotic cells. Moreover, phagosomes from BHK cells have also been shown to contain ER proteins (34).

We therefore tested whether incubation of BHK cells with latex beads would affect the release of newly synthesized sterols from the cells to serum acceptors. Cells prelabeled with [14C]cholesterol and pulse-labeled with [3H]acetate were incubated for 60 min in 20% serum-containing medium supplemented with 0.8-μm diameter latex beads in near saturating conditions (1:10 dilution of a 10% bead suspension). The efflux medium was collected and cholesterol and its precursors analyzed from the cells and medium as above. Surprisingly, we found that while the efflux of newly synthesized [3H]cholesterol was not affected upon incubation with the beads, the efflux of [3H]lathosterol was enhanced by −25% (Fig. 8). Furthermore, although newly synthesized zymosterol was very efficiently effluxed already in the absence of beads (see Fig. 6) the amount of [3H]zymosterol in the serum further increased by 26.9 ± 7.7% upon the phagocytic stimulus. Instead, the efflux of prelabeled [14C]cholesterol was not affected (data not shown).
cursors to both methyl-
domains. Thirdly, the release of newly synthesized sterol pre-
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terol immediately following its synthesis. Although the actual
structurally different sterols when studying the fate of choles-
precursors could be modulated differently from that of newly
readily effluxed. Finally, the efflux of newly synthesized sterol
polar zymosterol (due to its additional double bond) being more
were significant differences between individual precursors,
containing 0.8-
biosynthetic sterols to serum.
FIG. 8. Effect of incubation with latex beads on the efflux of
biosynthetic sterols to serum. BHK cells cultured and labeled as in
the legend to Fig. 6 were chased for 1 h in the presence of 20% serum
containing 0.8-μm diameter latex beads to stimulate ER-plasma mem-
brane contacts. The medium and cells were collected and lipids ex-
tacted and analyzed by TLC and HPLC. The percentage of biosynthetic
cholesterol and lathosterol effluxed in the presence (+) or absence (−)
of the beads is indicated. *, [3H]lathosterol efflux, − versus +, p < 0.05.

DISCUSSION

In this study, the separation of individual cholesterol precur-
sors from each other and from cholesterol enabled us to com-
pare their ratio and characteristics within cells as well as their
release to extracellular acceptors. These analyses revealed
firstly, that the efficiency of cholesterol synthesis from acetate
varied significantly between cell lines, with BHK cells contain-
ing severalfold more of sterol precursors than cholesterol even
after 1 h of acetate labeling. Secondly, the cellular partitioning
of sterol precursors may differ from that of cholesterol imme-
diately postsynthesis. This was suggested by the more rapid
recovery of newly synthesized lathosterol from membranes res-
sting detergent solubilization. The finding implies that latho-
sterol moves more rapidly than cholesterol within the cell to
reach sites enriched in cholesterol-sphingolipid-rich membrane
domains. Thirdly, the release of newly synthesized sterol pre-
cursors to both methyl-β-cyclodextrin and serum was greater
than that of newly synthesized cholesterol. Moreover, there
were significant differences between individual precursors,
zymosterol efflux being much greater than that of latho-
sterol. This correlates with sterol hydrophilicity, with the more
polar zymosterol (due to its additional double bond) being more
readily effluxed. Finally, the efflux of newly synthesized sterol precursors could be modulated differently from that of newly
synthesized cholesterol.

Our findings emphasize the necessity to accurately separate
structurally different sterols when studying the fate of choles-
terol immediately following its synthesis. Although the actual
amounts of cholesterol precursors present in the cells and in
the circulation are small their proportion of the newly synthe-
sized sterol pool may be substantial. As cholesterol precursors
apparently move about more freely than cholesterol, pooling of
data could result in the overestimation of the transport rate of
cholesterol and in misinterpretations regarding the insensitiv-
ity of cholesterol to perturbations that do affect the movement
of cholesterol but not that of its abundant precursor sterols.

The extractability of newly synthesized cholesterol and ste-
rol precursors varied considerably when cells were rapidly incu-
bated with methyl-β-cyclodextrin after different periods of
chasing. We have previously used cyclodextrin extractability of
newly synthesized cholesterol as a measure of its plasma mem-
brane arrival (27). Evidently, this cannot be directly used to
compare the plasma membrane arrival of cholesterol and its
precursors because the differential desorption of sterols from
the membrane (21) as well as differential affinity toward cyclo-
dextrin may contribute to the process. Yet, the time-dependent
changes in the cyclodextrin extractability of each sterol reveal
interesting differences, with newly synthesized lathosterol and
desmosterol behaving more similarly to cholesterol than zy-
mosterol (Fig. 3). The increasing release of newly synthesized
lathosterol, desmosterol, and cholesterol to cyclodextrin pre-
sumably reflects their increasing appearance in efflux acces-
sible membrane domains. On the other hand, zymosterol efflux
to cyclodextrin was initially very high but gradually decreased.
This could derive from the combination of inefficient choles-
terol synthesis, rapid zymosterol desorption from the mem-
brane and its eventual, relatively slow conversion to other
sterols. The data would be in accordance with the rapid deliv-
ery of zymosterol to the plasma membrane and slow metabo-
ism to cholesterol as reported by Lange et al. (19).

The use of serum as sterol acceptor has several important
distinctions compared with methyl-β-cyclodextrin. The efflux is
slower and consists of both diffusion and apolipoprotein-medi-
ated components (35). In addition, serum incubation is known
to activate physiological signaling and trafficking pathways
implicated in the efflux process. The preferential efflux of sterol
precursors compared with cholesterol also applied when using
serum as an acceptor. However, serum incubation enhanced the
efflux of newly synthesized cholesterol relative to the pre-
cursors especially in BHK cells. This is observed when compar-
ing the ratios of radiolabeled sterols in the medium at the same
chase time following either cyclodextrin extraction or serum
incubation (compare Fig. 3b and Fig. 6b). The finding supports
the idea that the efflux of newly synthesized cholesterol could
be regulated by HDL or other biological acceptors (20).

Consistently with the enhancement of cholesterol efflux by
serum, newly synthesized cholesterol was also more abundant
than the precursors lathosterol and zymosterol in the major
lipoprotein acceptors. According to the current view, small
pre-β migrating HDL particles are likely to serve as the initial
acceptors followed by further lipidation of HDL and gradual
transfer of the sterols to LDL (36). As the majority of sterols
recovered from LDL were free, i.e. unesterified sterols the
initial transfer did not seem to require the actions of the
plasma lipoprotein modifying enzymes lecithin:cholesteryl acyl
transferase and cholesteryl ester transfer protein. The low
levels of cholesterol precursors in normal human serum com-
pared with the levels that appear to be produced by tissues
probably reflect efficient secretion of precursors into the bile as
well as their conversion to cholesterol (7, 37).

Earlier studies have shown that the transport of newly syn-
thesized cholesterol is relatively resistant to Golgi disassembly
(27, 38, 39). However, the molecular machineries operating in
this Golgi-bypass route have remained elusive. In the present
work, we found that pharmacologically induced actin polymer-
ization inhibited the release of newly synthesized cholesterol
to methyl-β-cyclodextrin, and that this effect was additive with
the inhibitory effect of BFA. Although quantitatively minor,
this efflux inhibition provides the first indication for a potential
involvement of the actin cytoskeleton in the Golgi-bypass route
of cholesterol transfer. Moreover, actin may also play a role in
the mobilization of sterol precursors as similar inhibition was
observed for lathosterol efflux upon enhanced actin polymeri-
zation. Whether newly synthesized cholesterol and lathosterol
use the same Golgi-bypass mechanism remains open. The more
rapid association of lathosterol with DRMs and the enhance-
ment of lathosterol but not cholesterol efflux upon stimulation
of phagocytic uptake would nonetheless argue for a more re-
stricted mobility of cholesterol.

In using latex bead internalization as a means to promote
ER-plasma membrane contacts we cannot rule out the possi-
bility that the enhanced lathosterol efflux is due to some other reorganization in the cell upon phagocytic uptake. However, direct fusion of ER subdomains with the plasma membrane could potentially provide a rapid means to transfer precursor sterols between membranes, allowing them to bypass the cytoplasmic environment. Newly synthesized cholesterol is thought to be delivered to the plasma membrane by vesicular transport (38, 39) and may not be able to employ such a pathway. Interestingly, Patterson et al. (40) found that aspirin-induced redistribution of F-actin into a tight cortical layer subjacent to the plasma membrane prevented coupling between ER and plasma membrane Ca\(^{2+}\) entry channels. It could be that actin-dependent ER-plasma membrane transfer may also operate in the case of some of the sterol precursors.

An intriguing question raised by earlier studies and reinforced by our work is the apparent “leakiness” of the cholesterol biosynthetic system. The rapid cellular transfer and efficient desorption of sterol intermediates facilitate their removal to lipoprotein acceptors. However, this seems poorly compatible with the complexity and high energy expenditure of cholesterol biosynthesis. It is possible that delivery of biosynthetic sterol with the complexity and high energy expenditure of cholesterol biosynthesis. It is possible that delivery of biosynthetic sterol to the plasma membrane by vesicular transport (38, 39) and may not be able to employ such a pathway. Interestingly, Patterson et al. (40) found that aspirin-induced redistribution of F-actin into a tight cortical layer subjacent to the plasma membrane prevented coupling between ER and plasma membrane Ca\(^{2+}\) entry channels.

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Sari Lusa, Sanna Heino and Elina Ikonen

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