59 Wirtz, K. W. A., Op den Kamp, J. A. F., and Roelofsen, B., Phosphatidylcholine transfer protein: properties and applications in membrane research, in: Progress in Protein-Lipid Interactions, vol. 2, pp. 221–265. Eds A. Watts and J. J. H. H. M. de Pont. Elsevier Science Publishers, Amsterdam 1986.

60 Yaffe, M. P., and Kennedy, E. P., Intracellular phospholipid movement and the role of phospholipid transfer proteins in animal cells. Biochemistry *22* (1983) 1497–1507.

0014-4754/90/060592-08$1.50 + 0.20/0

Intracellular sterol trafficking

M. P. Reinhart

Biochemistry and Chemistry of Lipids, USDA-ARS-ERRC, 600 E. Mermaid Lane, Philadelphia (Pennsylvania 19118, USA)

Summary. Sterols are acquired by cells either biosynthetically by the interaction of cytoplasmic and endoplasmic reticulum elements, or by endocytosis. The subcellular distribution of sterols, however, argues that sterols are trafficked quickly from sites of acquisition to target membranes, particularly the plasma membrane. The mechanisms mediating this movement might include aqueous diffusion, vesicles of either a unique pathway or of the protein secretory pathway, or carrier proteins. These mechanisms are discussed and the limited data concerning each are presented. Finally, a theory is proposed which describes how sterols and other membrane reinforcing molecules might have driven the evolution of intracellular membranes, thus establishing the dynamic membrane system of modern eukaryotes.

Key words. Sterol synthesis; cholesterol; plasma membrane; endoplasmic reticulum; sterol carrier proteins; bacteriohopanes.

Introduction

The connection between cholesterol and the appearance and progression of atherosclerosis has fostered a voluminous amount of sterol-related research. This research has led to our detailed understanding of the extracellular trafficking of sterols by lipoproteins and of lipoprotein uptake by receptor-mediated endocytosis[16]. In addition, the major regulatory enzyme of sterol biosynthesis in higher animals has been identified as 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) and this enzyme has been examined in detail. Still, our understanding of the cell biology of sterols remains in its infancy. For example, something as fundamental as the subcellular distribution of sterols remains in dispute[40, 119] as does the exact site of sterol synthesis[55, 84]. Further, little research has been directed toward the elucidation of the mechanisms which mediate the intracellular movement of sterols, although such movement would be expected to be important in fueling retrotransport of cholesterol to the liver for excretion which in turn could have favorable impact on atherosclerotic regression[81].

In this paper, it will first be documented that sterol trafficking does occur by demonstrating that sterol synthesis proceeds in a membrane which is not the major repository of cellular cholesterol. Several mechanisms by which the sterol might be trafficked between the site of synthesis and target membranes will then be described and the limited data available for each will be discussed. Finally, attention will turn from the question of 'how' sterol is trafficked to an equally interesting topic: why? This discussion will culminate with the presentation of a theory which suggests that the evolutionary development of sterols necessitated the co-appearance of sterol-poor intracellular membranes in order to preserve a site compatible with protein translocation.

Subcellular site of sterol biosynthesis

Chesterton[21] originally demonstrated that when radiolabeled mevalonic acid (a water-soluble intermediate in sterol biosynthesis) is injected into rats, radiolabeled sterol can be detected in liver tissue within 2 min. Analysis of the distribution of the label at early times after mevalonate administration revealed that radiolabeled squalene, lanosterol and cholesterol are all found in both the granular and agranular microsomal elements (rough and smooth endoplasmic reticulum (RER and SER)). These membranes were concluded to be the site of sterol biosynthesis.

Lange and Steck[57] however, questioned this conclusion, since the microsomal fraction of liver tissue contains organelle membranes other than RER and SER[108]. These include plasma, Golgi, and perhaps other membranes. In a series of experiments involving double label incorporation in order to distinguish old versus newly synthesized cholesterol, they demonstrated that newly synthesized sterols are not found in the ER fraction. In an alternative approach, we[84] and others[42, 66] analyzed the location of the microsomal enzymes involved in sterolgenesis rather

than of intermediates, in sucrose density gradients. We found that all of the requisite membrane bound enzymes of sterol biosynthesis in the microsomal fraction distributed with the SER and RER. Therefore, we must face the paradoxical situation in which the enzymes of sterol biosynthesis are located in ER membranes while intermediates and cholesterol itself, all highly water insoluble, might not be.

More recently, Lange and Muraski [55] have demonstrated the topographic heterogeneity of sterol biosynthesis. They reported that not only do the intermediates of sterol biosynthesis not all equilibrate with the enzymes of sterolgenesis in density gradients, but they do not equilibrate with each other. Squalene, lanosterol and cholesterol were each found at distinct density regions of sucrose gradients. We have also found that this is true when sterol synthesis from mevalonate is carried out in vitro by purified stripped RER membranes (Reinhart, unpublished data). Intermediates, particularly squalene, do not equilibrate in gradients with the biosynthetic enzymes. Taken together with data from other laboratories we believe that the paradox can now be explained. Bloch and co-workers [33, 35, 71, 88, 101, 106] first characterized a protein called supernatant protein factor (SPF) and he as well as others [36, 90–93, 98] demonstrated that this protein is required for the conversion of squalene to lanosterol. The protein, however, is soluble, which is interesting considering that it facilitates the conversion of a hydrophobic substrate to a hydrophobic product. Even more interesting is the requirement for phospholipids [101] to stimulate SPF (also known as SCP1). Not only are there cytoplasmic proteins known to participate in sterolgenesis, but hydrophobic intermediates themselves might also be cytoplasmic. Loud and Bucher provided the first strong evidence of at least 2 separate pools of squalene in rat liver cells [61]. In his fractionation studies, Chesterton noted that some sterol intermediates, particularly squalene, are partially recovered in the soluble fraction [21]. Taken together, this information suggests that the pool of squalene undergoing active conversion to lanosterol might not be 'in' the membrane but rather 'on' it as part of a complex containing phospholipids, other sterol intermediates and specific proteins such as SPF which mediate the process. This hypothesis is shown diagramatically in figure 1.

Another approach to the localization of sterolgenic enzymes is by immunocytochemistry. Unfortunately, antibodies are currently available against only one major enzyme of sterolgenesis: HMG-CoA reductase. Several groups have used this approach to demonstrate the presence of HMG-CoA reductase on ER and special smooth membranes (crystalloid ER) found in some cells [22, 59, 96]. These anti-reductase antibodies have also led, however, to the unexpected finding that HMG-CoA reductase is present in the luminal compartment of peroxisomes [48, 49]. These studies sparked the search for the activity of HMG-CoA reductase and of other cholesterol

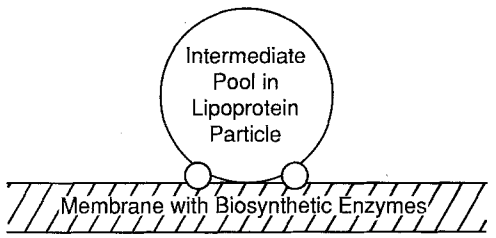

Figure 1. Possible interaction between membrane and cytoplasmic elements in sterol synthesis. Cellular fractionation studies have established that sterol biosynthetic enzymes are present on endoplasmic reticulum membranes [66, 84] but that the biosynthetic intermediates might not be [55, 57]. The presence of squalene, lanosterol and cholesterol ester in the cytoplasm [21], the requirement of a cytoplasmic protein, SCP1 for squalene to lanosterol conversion [35, 36], and SCP1's requirement for exogenous phospholipid for activity [101], suggested that sterol synthesis might be a cooperative process between the biosynthetic membrane (ER) and a cytoplasmic lipoprotein-like particle which contains the intermediates. Presentation of the intermediates for conversion could be under the mediation of SCP1, SCP2 or other accessory elements, as indicated by the small circles.

biosynthetic activities in peroxisomes and this has now been confirmed by two groups [2, 109]. While it is clear that cholesterol biosynthesis can proceed in the peroxisome, particularly in animals in which sterol biosynthesis has been induced by the bile acid sequestrant cholestyramine, the physiological relevance of this finding is not yet known.

In addition to biosynthesized cholesterol sterols can also be acquired through endocytic processes. Since cells are capable of utilizing this sterol, it is inferred that the sterol is trafficked from lysosomes to sterol target membranes. Niemann-Pick type C is a genetic disorder in which free sterol can not be released properly from lysosomes, resulting in its build up in that compartment [60, 79]. Analysis of the biochemical basis of this disorder should prove invaluable for the elucidation of trafficking of sterol from the lysosomal compartment.

Subcellular distribution of sterols

The synthesis of sterols in the ER would suggest that this membrane might be relatively sterol rich. In order to measure sterol content of the ER and of the other cellular membranes, a number of laboratories have employed a variety of techniques including cell fractionation, rapid plasma membrane isolation, enzymatic labeling and ultrastructural localization. Far from demonstrating a high sterol content in the ER, these studies have indicated that the plasma membrane is the membrane with the richest sterol content.

Using gradient density centrifugal techniques, Coleman and Finean [24] prepared plasma membrane fractions from a number of guinea pig tissues. A common property of these membranes was the high cholesterol/phospholipid (c/p) ratio (roughly equimolar) relative to whole cell values. This observation was extended to rat liver plasma membranes by Pfleger et al. (c/p of 0.74) [80]. Having earlier noted a nucleo-cytoplasmic distribution of both 5′-

nucleotidase and cholesterol, Thines-Sempoux et al.[108] combined isopycnic density gradient centrifugation with a density shifting technique which they devised. When mixed with the detergent digitonin, it was noted that the activity of the plasma membrane marker enzyme 5'-nucleotidase was shifted to higher density in gradients[1]. Other microsomal marker enzymes, however, were not shifted by this treatment. Analysis of the cholesterol distribution across density gradients of digitonin treated microsomes revealed that the majority of cholesterol was also shifted. The conclusion from these studies was that not only does the plasma membrane have a high c/p ratio but the ER must contain very low c/p ratios.

Colbeau et al.[23] used established techniques to purify a number of rat liver organelles for lipid analysis, thus extending our knowledge of cholesterol content to a number of other cell structures. The purity of these membranes was assessed by marker enzyme analysis. Plasma membrane, exhibited the highest sterol content with a c/p ratio of 0.76. Outer mitochondrial membrane c/p ratio was determined to be 0.12, while cholesterol was virtually undetectable in the inner membrane. A c/p value of 0.24 was found for the SER but a portion of this sterol might actually have been due to contamination with plasma membranes, as indicated by marker enzyme analysis. RER, a membrane known to contain cholesterolgenic enzymes, contained very low sterol levels; c/p = 0.06. Mammalian nuclear envelope membranes resemble ER membranes in their low c/p ratios: 0.104[51]. Interestingly, nuclear membranes exhibited a four-fold higher sterol ester content than microsomes[51]. Similar sterol/p values have been demonstrated for other tissues and in the tissues of other animals[25,94], lower eukaryotes[113], and plants[43,95]. Thus, classical cell fractionation has provided good evidence that plasma membranes are sterol rich while many internal membranes, including the sterol biosynthetic membranes themselves, are relatively sterol poor.

Another technique which demonstrated the sterol-rich nature of the plasma membrane is the DEAE bead technique devised by Gotlib and Searls[38] and modified by DeGrella and Simoni[28]. When suspended cells in culture are mixed with DEAE beads, they rapidly bind to the beads by virtue of plasma membrane charge. Upon application of shear force, cells are ruptured and their internal contents are released. The plasma membranes, however, remain adherant to the beads and can be easily recovered in purity sufficient to allow lipid analysis. When applied to CHO cells, DeGrella and Simoni[28] found a c/p ratio of 0.84, similar to the values obtained above and in good agreement with values obtained from erythrocytes[117] where c/p determination is not complicated by the presence of internal membranes.

A novel approach to the analysis of cholesterol distribution, the enzymatic modification of plasma membrane cholesterol[39], was used by Lange and Ramos[56]. In this technique, cells are exposed to the enzyme cholesterol oxidase under conditions which allow the plasma membrane cholesterol to be oxidized to cholestenone (low ionic strength buffer and glutaraldehyde). The incubation period is of sufficient time to allow inner leaflet sterol to flip to the outer leaflet for oxidation (half-time of 3 s determined using this technique[53]). 94 %, 92 % and 80 % of cellular cholesterol was converted in fibroblasts, Chinese hamster ovary cells or hepatocytes, respectively. These figures reflect the proportion of total cellular cholesterol found in the plasma membranes. Van Meer, however, used model calculations to suggest that these figures do not correctly reflect plasma membrane cholesterol content, but rather, that most of the cell cholesterol is found on internal membranes[119]. These calculations are based on c/p ratios of specific membranes and the known surface area of the membrane obtained by morphometric analysis (e.g.[14]). In MDCK strain II, for instance, the plasma membrane surface area (both leaflets together) is approximately 2464 μm^2. These cells contain an average of 6×10^9 cholesterol molecules, which, at 37 $Å^2$ per sterol molecule, would cover 2220 μm^2. If 90 % of this cholesterol was present in the plasma membrane, 1998 μm^2, or 81 % of the plasma membrane surface area would be taken up by sterol. Using the commonly reported c/p value of 0.8, van Meer argued that cholesterol would cover only 31 % of the plasma membrane surface area and would account for only 34 % of the total cellular cholesterol. Similar arguments were made for other cell types including rat liver cells and BHK cells[119]. Recent work by Brasaemle et al.[15] suggests that estimates of plasma membrane cholesterol obtained using the above-described cholesterol oxidase procedure might be spuriously high due to perturbations of the membrane during treatment. Inclusion of magnesium ions slows observed flip-flop rates of cholesterol to a half-time of 50–130 min. Thus, in the absence of sufficient magnesium ions, the oxidase might gain access to the interior surface of the membrane and perhaps to intracellular membranes. Recently, however, data were obtained from dye-binding and two-phase aqueous partition studies which confirm the high proportion of total cellular sterol in the plasma membrane[58].

Filipin, a polyene antibiotic, has been used to probe cellular sterol distribution at both the light microscope level, by virtue of its fluorescence[9], and at the ultrastructural level[4] by virtue of pit formation which it induces. The frequency of pits formed in the presence of filipin is a function of sterol concentration in that membrane. Pit formation requires long incubation times making it difficult to rule out sterol redistribution. In addition, its binding can be diminished by the presence of certain coat proteins like clathrin. Other cholesterol-specific stains would be valuable for further analysis of subcellular distribution of sterol.

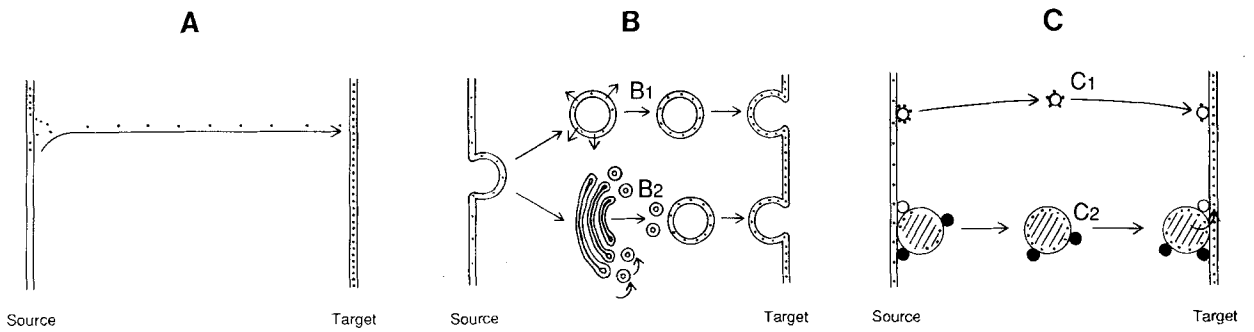

Figure 2. Possible mechanisms of intracellular sterol trafficking. Aqueous diffusion is diagrammatically represented in panel A. In panel B, B1 represents a unique vesicle-mediated trafficking system. The enrichment of cholesterol seen in target membranes could be achieved by removal of phospholipid from such vesicles (arrows). Vesicular trafficking of sterol also could be achieved by elements of the protein secretory pathway (B2). Carrier-mediated trafficking (panel C) might be accomplished by specific sterol carrier proteins (C1) or via lipoprotein-like particles of the cytoplasm (cytolipophorins[85]), as in C2. This highly speculative latter mechanism might suggest an intracellular ligand, receptor mechanism of delivery analogous to the extracellular system.

Modes of sterol trafficking

The separation of the site of synthesis and the major cell repository of sterol necessitates trafficking. Little is known about the exact mechanisms used by cells, but several mechanisms have been proposed (fig. 2): 1) aqueous diffusion and differential partitioning; 2) vesicle-mediated transport; and 3) carrier-mediated transport[40]. Before describing these mechanisms in more detail, it is important to point out that more than one mechanism might be at work in the cell, perhaps a different mechanism for each trafficking route. In addition, any one route might utilize one or more of these (or additional) mechanisms to complete the trafficking process.

Aqueous diffusion and differential partitioning

When unilamellar vesicles of differing lipid compositions are mixed, transfer of lipids between the two types can be observed[65]. The transfer of lipid between vesicles could occur by two possible mechanisms: 1) transfer during the formation of collision complexes or, 2) diffusion of lipid from donor to acceptor membranes through the aqueous phase. McLean and Phillips[65] utilized a model system of negatively charged cholesterol donor vesicles and neutral acceptor vesicles. No protein was present in the assays. The donor membranes are easily separated from the acceptor vesicles by passage over a DEAE-Sepharose column which selectively retains the donor by virtue of its charge. This allows the amount of radiolabeled sterol transferred to be quantified. That fusion of vesicles did not occur during the course of the incubation was confirmed by electron microscopy. During a 12-h incubation at 37 °C, 90 % of the donor vesicle cholesterol was transferred to acceptor vesicles. The process was first order with a $T_{1/2}$ of 2.3 ± 0.3 h. When donor and acceptor vesicles were separated by a dialysis membrane, transfer became slow but the rate was in accord with Fick's 1st law, supporting aqueous diffusion and not collision as the mode of transfer. The rate-limiting step was identified as desorption from the donor membrane. Thus, it is conceivable that intracellular sterol trafficking is accomplished by desorption of newly synthesized sterol from the biosynthetic membrane followed by aqueous diffusion to target membranes (fig. 2, panel A). Because of the variability in sterol content between the different organelles, a mechanism to explain the differential partitioning of sterol must be determined. Wattenberg and Silbert[122] examined the partitioning of sterol into isolated organelle membranes and into vesicles whose lipid contents were reconstituted from purified organelles. They observed that sterol partitioned into plasma membrane with the greatest affinity followed by membranes of ER and mitochondria. The same was true when reconstituted acceptor vesicles were generated from purified organelles. Particular importance was assigned to sphingomyelin and unsaturated fatty chains in causing this affinity for sterol. The qualitative pattern of sterol partitioning followed the order in which it is found in cell membranes. Quantitatively, however, the partitioning was not to the same degree as seen in vivo. It is improbable, therefore, that the sole mediator of intracellular sterol transport is aqueous diffusion.

Vesicle-mediated transport

A hallmark characteristic of eukaryotes is the presence of an extensive network of intracellular membranes. Many of these membranes communicate with one another through vesicular exchange. A well-characterized and much studied example is the exocytic pathway, which Cavalier-Smith[17] has argued was the driving force in eukaryotic evolution. Membrane and secretory proteins are synthesized on the RER, packaged into smooth membrane vesicles and moved to the Golgi complex[120]. Here, fusion with a cis-Golgi element exposes nascent proteins to Golgi enzymes for further processing. Through a series of vesiculations, nascent proteins are carried through consecutive elements of the Golgi until processing is complete. The resulting secretory vesicles

fuse with the plasma membrane resulting in the release of soluble contents and the delivery of plasma membrane proteins to their target. Since sterols are also generated on the ER, it is appealing to speculate that they move to the plasma membrane in concert with nascent proteins. Alternatively, an entirely separate system of vesicles might transfer sterol to the plasma membrane without intermediate stopover at the Golgi. Such a system has been identified to deliver phospholipid to the plasma membranes of *Dictyostelium discoideum*[31].

Support for vesicle-mediated sterol transport has come from two laboratories which examined kinetics of appearance of newly synthesized cholesterol at the cell surface. Using the DEAE-bead technique which was described above, DeGrella and Simoni[28] examined the kinetics of appearance of newly synthesized [^{3}H]cholesterol at the surface of CHO cells. After a short lag, cholesterol arrived at the plasma membrane in linear fashion at a rate which paralleled the rate of synthesis (indicating that the plasma membrane is the major target for cholesterol in these cells). A $T_{1/2}$ for delivery was about 10 min, indicating that delivery was too rapid to be accounted for by the aqueous diffusion model. The transport mechanism was shown to be temperature sensitive in these cells. While synthesis proceeded at 15 °C, delivery to the plasma membrane was curtailed. Energy poisons (carbonyl cyanide p-chlorophenylhydrazone, KCN and KF) also inhibited delivery, but maximal inhibition was achieved only by using combinations of these drugs. Treatment with the poisons did not result in a loss of sterol from the plasma membrane, indicating that energy expenditure is not necessary to maintain the high concentration of sterol in that membrane[28].

When these investigators treated cells with [^{3}H]mannose in order to examine the time course of glycoprotein synthesis and delivery to the plasma membrane, a long lag (20–30 min) followed by a slow increase was observed. Since cholesterol delivery was much faster than this, it was argued that cholesterol delivery probably did not involve passage through the Golgi compartment. Wieland et al.[125] however, demonstrated that proteins traverse the Golgi with a $T_{1/2}$ much longer than the rate of bulk flow through that compartment as measured with the tri-peptide glycosylation substrate Asn-X-Ser/Thr. Following glycosylation, which indicated arrival in the RER lumen, this compound was secreted with a $T_{1/2}$ of 10 min, or, roughly the same as noted for cholesterol in the above experiment. Thus, sterol moves to the plasma membrane with kinetics similar to the rate of bulk flow in the exocytic pathway and might, therefore, be trafficked by that pathway. Strong evidence against the involvement of Golgi in the flow of sterol to the plasma membrane from the ER was obtained by Kaplan and Simoni[47] who noted that a potent inhibitor of exocytosis, monensin, was without effect on cholesterol trafficking. Thus while kinetics demonstrate that sterol is delivered to the plasma membrane in the same time frame as bulk flow through the Golgi, passage through the Golgi is questioned due to the lack of influence of monensin. If sterol is trafficked via a vesicular route, it is very likely that they take a route distinct from that followed by secretory and plasma membrane proteins.

A vesicular trafficking system has also been postulated by Lange and Matthies[54] who studied cholesterol trafficking in human fibroblasts using the cholesterol oxidase technique described earlier. Newly synthesized (radiolabeled) cholesterol becomes a substrate for this enzyme only as it arrives at the plasma membrane. While these investigators also observed first order kinetics. The $T_{1/2}$ of delivery was 1–2 h, or only slightly more rapid than would be expected if movement was due to aqueous diffusion. Interestingly, no difference in delivery rate was observed when cells in various stages of growth or nutritional states were examined.

If a vesicular mode of transport is used to traffic sterol to the plasma membrane, one is left with the problem of how the low c/p ratio of the source membrane is modified to the high c/p ratio of the plasma membrane. This could be accomplished either by a mechanism which preferentially delivers the sterol, or by a mechanism which removes phospholipid from trafficking intermediates (as shown schematically in fig. 2, panel B1) or from the plasma membrane. Phospholipid transfer proteins are known to be present and might mediate such a phenomenon[10,11,26,29].

Carrier-mediated sterol transport

A final mechanism to be considered is the carrier-mediated trafficking of sterol through the cytoplasm. This could be accomplished by sterol carrier proteins, as illustrated in figure 2, panel C_1, or, as we have recently speculated, by lipoprotein-like structures in the cytoplasm (fig. 2, panel C_2). Examples of cytoplasmic proteins which bind and carry steroids with a high degree of specificity are well known. Steroid hormone receptors[70] and the 25-hydroxy cholesterol binding proteins[44,45,103–105], for example, have been well characterized. It has been widely assumed that sterol binding proteins would exhibit properties similar to the steroid binding proteins, especially the ability to bind to sterols with high specificity and affinity. Many of the proteins which have been identified by their ability to bind radiolabeled cholesterol, however, are probably not involved in transporting cholesterol, but, rather, bind cholesterol non-specifically through interactions with hydrophobic domains[41,99,100].

The ability to carry sterols has been inferred to be a property of a group of proteins, collectively known as sterol carrier proteins, because of their influence on in vitro cholesterol biosynthetic reactions[92,93]. Squalene and sterol carrier protein, or SCP, was originally thought to be involved in cholesterol metabolism[86] and while this contention has not been entirely ruled out[30], this protein has proven to be identical with Z-protein, also known as

fatty acid binding protein[37]. A second protein[69], sterol carrier protein one (SCP1), also known as supernatant protein factor[33], appears to be a necessary participant in the conversion of squalene to lanosterol. This activity also requires exogenous phospholipids, supporting the model of synthesis diagrammed in figure 1, which suggests that a cytoplasmic lipid particle interacts with SCP1 to mediate proper orientational presentation of squalene to other enzymes in the endoplasmic reticulum. Actual binding of cholesterol to the protein has not been demonstrated, suggesting that SCP1 is a misnomer. It appears, however, that it is necessary for the proper trafficking of at least one intermediate through the biosynthetic pathway.

A third protein was identified by its impact on a number of cholesterol biosynthetic reactions, particularly in the conversion of lanosterol to cholesterol[91]. This protein, sterol carrier protein two (SCP2), appears not to be absolutely necessary for, but highly stimulatory to, these reactions. SCP2 has been shown to be identical to nonspecific lipid transfer protein, previously isolated and characterized[5,10,69,110]. The tissue distribution of this protein also suggests that it is involved in cholesterol synthesis and steroidogenesis, the largest amounts being found in liver (0.78 μg/mg) followed by intestine (0.46 μg/mg). High concentrations are also seen in steroidogenic tissues but most other cell types have relatively low amounts (0.1 μg/mg)[107].

SCP2 has been purified from a number of sources including rat liver[5,10,110,115], bovine liver[26], goat liver[3], human liver[116] and rat ovary[102]. The SCP2's isolated from bovine and rat liver show greater than 90 % sequence homology[67,123]. We have recently isolated from chicken liver a 12,500 Da basic protein which reacts on Western blots with antibodies directed against rat SCP2. The amino acid composition of this avian protein, however, is quite dissimilar to the bovine and rat SCP2's. Sequence and activity information regarding this protein should prove invaluable to understanding its structure and function (Reinhart and Avart, unpublished observations). SCP2 is a small protein, M_r 12,500 Da, but it has previously been suggested that it is made as a 14 kDa precursor protein which is subsequently trimmed[111]. A cDNA for rat SCP2 which contains the entire SCP2 sequence, has recently been obtained and its structure suggests that it is initially made as a 29 kDa precursor[7]. In vitro translation products support the synthesis of a 29 kDa protein.

A curious but consistent result of antibody production against SCP2 is the identification of a 55–58 kDa protein which is immunologically cross-reactive[50,107,111,112]. Van Amerongen et al.[116], in their study of liver samples from patients with Zellweger's syndrome, noted the virtual absence of both 12.5 kDa SCP2 and the 55 kDa SCP2-like protein. Further it was deduced from an analysis of normal liver that the 55 kDa form is restricted to the peroxisomes while the low molecular weight form is cytoplasmic[118]. More recent studies have demonstrated the presence of both species in peroxisomes[112]. That the 55 kDa protein is related to SCP2 is supported by SCP2 sequence data[67] which discloses the presence of the known peroxisome targeting sequence ala-lys-leu at the carboxyl terminus. Recent studies suggest that the majority of SCP2 is found in peroxisomes[50,112]. The physiological importance of this finding is not known, but could be related to the recently discovered ability of the peroxisome to synthesize cholesterol and perhaps bile acids[52].

Another site where SCP2 is frequently noted using immunological probes, is on the inner mitochondrial membrane. This is the site of side-chain cleavage as a prelude to bile acid and steroid hormone production. Chanderbahn et al.[18–20,114] has implicated SCP2 in steroidogenesis. Subcellular localization therefore supports the notion that a possible role of SCP2 in the trafficking of cholesterol is in the diversion of sterol from a default pathway which would otherwise take it to the plasma membrane, to the inner mitochondrial membrane for further metabolism. Analysis of SCP2-cDNA suggests that SCP2 is made as a larger precursor protein, and further, that among the elements removed during post-translational processing of SCP2 are sequences which share many features in common with known mitochondrial targeting sequences (J. T. Billheimer, personal communication). This opens the possibility that a form of SCP2 is directly recognized by and transported into the mitochondrion.

The mode of action of SCP2 is unknown but it could function by: 1) causing fusion of membranes; 2) influencing the off-rate of sterols from membranes; 3) acting as a carrier by binding cholesterol directly; 4) bringing two membranes into apposition, allowing the intermingling of outer leaflet lipids without causing fusion. In vitro assays have demonstrated the ability of SCP2 to facilitate transfer of a wide variety of polar lipids between membranes[10–12,18,26,69,110]. It, however, does not cause the transfer of triglycerides and cholesterol esters from the liposomal core. This has been used as evidence that SCP2 does not act by causing membrane fusion. Highly purified SCP2 preparations do not contain cholesterol[18] nor does purified SCP2 bind cholesterol in in vitro experiments[115]. Thus its name 'carrier protein' might be a misnomer. Rather than acting as a carrier, a more likely mechanism for the action of SCP2 is in bringing together two membranes to allow intermingling of outer leaflet lipids. It has been noted that it is an amphipathic molecule[29,77] with the termini being positively charged. Thus, it could interact with two negatively charged membranes, pulling them close together for sterol (and other lipid) exchange[115]. Much work remains to be done to elucidate SCP2's true physiological role and mode of action.

The cytoplasm of cells is known to contain lipid, including cholesterol, in the form of lipid droplets. Loud and Bucher[61] identified at least two pools of cellular squalene

and Chesterton[21] demonstrated that at least one pool is cytoplasmic. In addition he found cholesterol ester present in the cytosolic fraction. In a preliminary report, it has been demonstrated that at least a portion of the cytoplasmic lipid of rat liver is not in low density lipid droplets, but in particles which behave in density gradients as high density lipoproteins[85]. Arguably, these could in fact be contaminating serum lipoproteins, however, such lipoprotein-like particles have also been detected in plant cytoplasm and the cytoplasm of a number of unicellular eukaryotes[85]. Thus, it is conceivable that cholesterol is trafficked by a lipoprotein-like particle between source and target membranes in the cell. An ideal candidate for the up- and down-loading of such a particle would be SCP2. The data presented above which supports vesicular transport[28,47,54] would also support such lipoprotein-like transport. Carrying the analogy to serum lipoproteins a step further, one could postulate that these lipoproteins have ligands specific for receptors found on various targets and that the receptive state of these receptors is mediated by the lipid environment, thus regulating delivery (fig. 2, panel C_2). Obviously this is a highly speculative suggestion, but one worthy of further research.

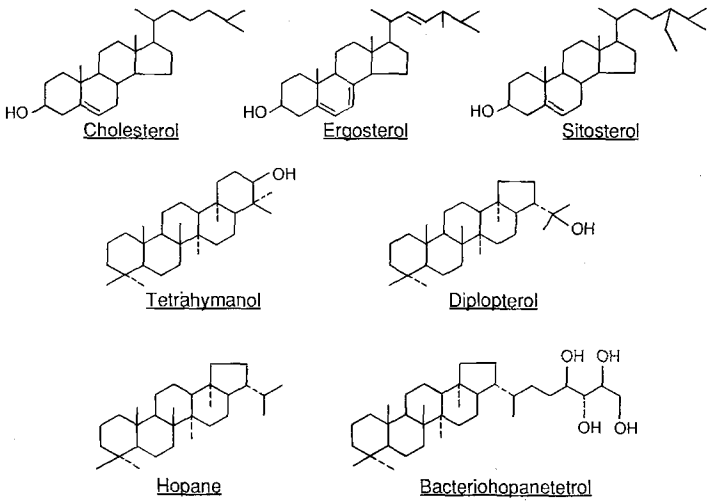

Figure 3. Structures of membrane reinforcing molecules. Structures of several major membrane sterols as well as the quasi-hopanoid tetrahymanol and the true hopanoid diplopterol are shown. Also shown is the 'molecular fossil' hopane and bacteriohopanetetrol, a commonly found hopane of diverse modern aerobic prokaryotes[87].

Sterols and membrane dynamics

Sterols are nearly ubiquitous among eukaryotes while almost universally absent from prokaryotes. The selective advantage imparted to eukaryotes by sterols is probably due to the impact on the structure of the plasma membrane, based on its relative abundance there. Why does the cell separate the site of sterol synthesis from its ultimate membrane destination, that is, why is sterol trafficked so specifically? Does this separation in any way influence membrane dynamics within the cell? The remainder of this paper will explore these questions by considering the effects of sterols on membrane properties and by analyzing the evolution of sterols and intracellular membranes.

Sterol impact on membrane structure

The insertion of sterols into phospholipid bilayers has profound impact on the properties of the bilayer. These have been summarized by Bloom and Mouritsen[13] as: 1) the elimination of the gel-liquid phase transition, 2) the retention of fluid-like characteristics over a wide temperature range, 3) the retention of a fluid-like microviscosity over a wide temperature range, 4) the reduction of area compressibility resulting in greater cohesivity and reduced permeability and, 5) increasing membrane order, thus causing membrane condensation and increased bilayer thickness. While cholesterol might be the most efficient sterol in causing the above properties[126], many other sterols will elicit similar membrane properties. It has been demonstrated that in order to have the maximum sterol-like effects on a membrane, sterol molecules should have three basic properties: a) a 3β-OH group; b) a planar four-ring system with projections only from the β face; and c) a hydrophobic 'tail'[126]. The major membrane sterols of animals, fungi and plants indeed share these properties (compared in fig. 3).

These benefits which are imparted to eukaryotic membranes have long been assumed not to be enjoyed by prokaryotes, since they do not contain sterols. Molecular paleontologists, however, noted the presence of pentacyclic triterpenoids in crude oils, oil shales and coal deposits greater than 500 million years old and reasoned that these 'hopanes' (fig. 3) might represent molecular fossils of currently existing molecules[75,82]. Analysis of a diverse spectrum of prokaryotes has, in fact, confirmed the widespread presence of bacteriohopanols[87]. The structure of a common member of this family is shown in figure 3. In a number of respects, the bacteriohopanols are similar to sterols. They are multiple-ringed structures with a side chain and, like sterols, are rendered amphipathic by hydroxyl moieties. Thus, bacterial membranes might benefit from 'sterol-like' effects due to the presence of bacteriohopanols or other membrane reinforcers. Some fundamental differences from sterols must, however, be considered. Most obvious is the fact that hopanes are pentacyclic rather than tetracyclic. Another striking difference is that the hydroxyl groups which render the hopane amphipathic are located on the side chain rather than the A ring. This indicates that hopanes would orient in phospholipid bilayers with the side chain toward the outside of the membrane rather than the interior. Finally, the methyl groups which project from the ring system do so from both the α and β faces. While sterol intermediates also contain projections from the α face, these are not present on any major mature mem-

brane sterol. Therefore, it would be expected that hopanols would exert a condensing effect on bacterial membranes but that this effect would not be as dramatic as that imparted to eukaryotic membranes by sterols. Indeed, initial investigations into physical and chemical properties have indicated that hopanols influence gel-liquid phase transition, maintain fluid-like properties, create fluid-microviscosity, give greater cohesivity and reduced permeability and cause membrane condensation [8, 46, 76]. The extents to which these effects are elicited by hopanols, however, are less dramatic than those elicited by the major sterols [76].

Not all prokaryotes contain hopanols. In those which do, they appear to be essential [34]. In many of those which do not, other membrane 'reinforcers' are present including bicyclic retinols. Interestingly, these molecules are all derivatives of terpenes [76]. Based on an apparent sequential phylogenetic appearance of terpene-derived membrane-reinforcing molecules, Ourrison et al. [76] developed an appealing scheme for the evolution of squalene cyclizing enzymes. There are examples of bacteria in which no membrane reinforcer has yet been identified, but it is believed that further scrutiny will reveal such molecules.

Any hypotheses concerning the role of sterols in membrane dynamics in eukaryotes must be tempered, therefore, by a consideration of similar roles performed by bacteriohopanols and other membrane reinforcers in bacteria. But what are the roles of sterols in plasma membranes, and if both prokaryotes and eukaryotes contain 'reinforcer' rich outer membranes, what drove the development of sterol poor intracellular membranes which are free of sterols, but which, paradoxically, synthesize sterols? Perhaps a more pertinent way in which to ask the question is: what selective advantages were imparted by membrane reinforcers to those organisms which first developed them during the course of evolution? An hypothesis is presented here which relates the development of effective membrane-reinforcing molecules with the evolution of cells with intracellular membranes devoid of those same molecules. This hypothesis is shown diagramatically in figure 4.

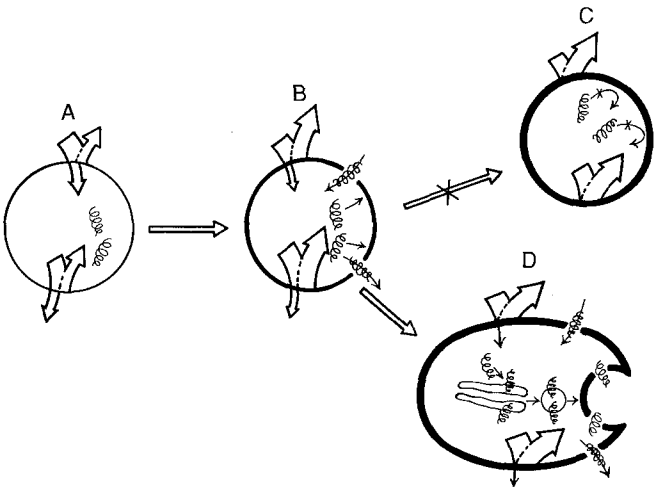

Figure 4. Possible role of membrane reinforcing molecules in guiding the evolution of the dynamic membrane system of modern eukaryotes. The appearance of primitive membranes (A) in the 'primordial soup' would have been of great selective advantage since this membrane would have allowed locally higher concentrations of substrates and of primitive enzyme systems, thus driving sequential reactions faster than in the uncontained, dilute 'soup'. To take maximum advantage of external nutrients and to expel toxic byproducts, the primitive lipid membrane would have been, by necessity, relatively permeable. The evolution of an efficient mechanism to insert proteins into membranes, plus the evolution of transport properties by these proteins (B) would have reduced the need for a high degree of permeability of the lipid aspect of the membrane. In turn, this could have fostered the appearance of the first membrane reinforcing molecules, which condensed and thickened the membrane (shown by line thickening) thus reducing permeability. The evolution of membrane reinforcers which virtually eliminate the permeability of the lipid aspect would put the regulation of the internal milieu totally under control of transport proteins (C). Such tight membranes, however, may have proven disadvantageous. In fact, one consequence of membrane reinforcement might be the prevention of spontaneous membrane protein insertion. Thus, increasingly efficient membrane reinforcement may have led to a bottleneck at B. This bottleneck could be removed if protein translocation is preserved on a membrane of low reinforcer content. Thus, the evolution of sterols, which are highly efficient phospholipid bilayer condensing agents, might have necessitated the co-evolution of a specific low cholesterol intracellular membrane, the endoplasmic reticulum, as a protein translocatory site (D). A separation of protein translocation site from the permeability barrier would therefore represent an alternative route to get around the bottleneck at B. Introduction of the transport protein to a high sterol environment would occur by increasing the sterol content of the membrane after its insertion, possibly upon fusion with the plasma membrane. This might in fact activate the protein [13].

During prebiotic evolution, it is postulated that organic molecules were formed through the action of physical and chemical forces [72]. The products formed by these processes constituted a 'primordial soup' of organic products. The evolution of efficiently catalyzed interconversion of these molecules, however, awaited the development of a means to maintain locally high concentrations of substrates to drive conversion. This could have been accomplished by the appearance of the first membranes. Such lipid membranes would have acted as a barrier to free diffusion, thus retaining molecules in a limited volume for conversion by primitive enzymes. The appearance of the first membranes, however, might also have presented problems. For instance, molecules in the 'primordial soup' on the exterior of the membrane would have to cross the barrier in order for the primitive cell to make use of them. Any by-products of conversion which proved toxic to other enzymatically catalyzed processes would poison the 'cell' unless they could also cross the barrier in the opposite direction. The advantageous properties of the lipid membrane which allowed intermediates (and the primitive enzymes themselves) to be retained in a limited area during conversion, therefore greatly enhancing the overall rate at which primitive metabolic pathways proceeded, had to be balanced against the ability of the membrane to admit potentially useful molecules and expel toxic by-products. Thus, by necessity, the earliest membranes, would have been relatively permeable.

Another major landmark in the evolution of membranes would have been the introduction of a new aspect to

the membrane: protein. This would have occurred with the advent of a reliable mechanism for insertion of proteins into the hydrophobic bilayer, such as by the 'helical hairpin mechanism'[32], or other possible mechanisms[68,83,97,121,124]. Such membrane proteins would have imparted an extreme selective advantage on the organism if, once in the membrane, they 'catalyzed' the selective and directional transport of specific molecules. Cells endowed with such transport proteins would be able to take full advantage of extracellular nutrients while being able to more rapidly expel by-products. With the development of this ability, the cell was released from the constraints of a relatively permeable lipid bilayer. Therefore, the earliest membrane reinforcing molecules, such as bicyclic retinols, could appear. Hopanols, which are more efficient at membrane condensation probably did not arise until after the appearance of oxygen in the atmosphere since they have never been found in a strict anaerobe (although their synthesis does not require oxygen). This suggests that the evolution of modern membranes was driven by the co-development of increasingly efficient and selective transport proteins and increasingly less permeable lipid aspects. Combined, these properties provide greater control over the intracellular milieu.

Although it is tempting to postulate that the lipid aspect might continue to evolve until it is nearly impermeable, such a progression might also be disadvantageous. During the initial evolution of transport proteins, the spontaneous translocation of these proteins occurred in a relatively permeable membrane and possibly through direct interaction with membrane lipids as has been proposed[69]. The appearance of membrane-reinforcing molecules which rendered the lipid aspect of the membrane increasingly more impermeable to small molecules, surely would have impeded the translocation of much larger molecules such as transport or secretory proteins. Thus, modern bacteria may have balanced the degree of membrane condensation by relatively ineffective reinforcer molecules against the need to translocate membrane and secretory proteins efficiently. Assuming this is true, how have eukaryotes developed the more efficient membrane condensing sterols, while maintaining the ability to effectively translocate proteins? The evolutionary answer appears to have been a separation of the functions. The appearance of oxygen in the atmosphere was followed by the oxygen-dependent synthesis of sterols, whose ability to render phospholipid bilayers effective permeability barriers was far greater than their ancestral hopanol counterparts. Since the degree of membrane condensation afforded by sterols might preclude the plasma membrane from being active in protein translocation, this function could have been preserved on an intracellular membrane of low sterol content (and which has no need to be an efficient permeability barrier). This represents the very essence of eukaryotic existence: compartmentation. While it seems paradoxical that sterol should be synthesized on a membrane which is sensitive to its presence, it is probable that such a situation would result in the evolution of the most sensitive regulatory responses. Thus one would predict that the permeability barrier benefits would be reflected by a high sterol content in the plasma membrane, as has been documented. Conversely, one would predict from this hypothesis, that protein translocation would not occur primarily at the plasma membrane, as in prokaryotes, but on sterol-poor internal membranes, which has also been documented. This hypothesis provides a possible answer to the question 'why is sterol trafficked?'. Further, it suggests that aqueous diffusion as a sole mechanism is improbable since it would not be rapid enough to ensure maintenance of low sterol content in the ER. Any other of the mechanisms elaborated in figure 2 are compatible with the hypothesis.

From the preceding argument, one would suppose that eukaryotes would have elaborate means to adjust cholesterol synthesis to balance with plasma membrane growth and maintenance needs. This is indeed the case and is accomplished in large part by altering the amount of the major regulatory enzyme, HMG-CoA reductase at transcriptional, translational and post-translational levels[16,62,73,74,96,103,104]. Biosynthesis, however, is not the only source of sterol in many eukaryotic cells. Endocytosis, most notably of the receptor-mediated type in higher animals, can be a major sterol source[16]. One would, therefore, expect that mechanisms would be present to limit the amount of cholesterol taken up from outside the cell. Again, the down-regulation of LDL receptors, has been elegantly demonstrated to accomplish this[16].

Still, if excess cellular sterol would have adverse impact on the ability of the cell to synthesize and translocate membrane and secretory proteins, one might further postulate the presence of ER mechanisms to protect against the sterol. In fact, Reinhart et al.[84] have postulated that this is the role of the enzyme Acyl CoA:Cholesterol Acyltransferase (ACAT), an ER enzyme, which in at least some species (e.g., rat) is restricted specifically to the RER. This enzyme esterifies free cholesterol rendering it more stable in a highly hydrophobic domain. Therefore, by the action of this enzyme, it is believed that free cholesterol is removed from ER membranes and localized to the interior of cytoplasmic lipid droplets. In the lipid droplet, ester hydrolysis can lead to the formation of free cholesterol which again becomes substrate for ACAT. This cholesterol ester cycle is now firmly established and morphological details have been examined[64]. Even in a ciliated protozoon, *Tetrahymena,* which does not synthesize sterols, but rather a quasi-hopanoid, tetrahymanol, and a true hopanoid, diplopterol (fig. 3), the enzymatic machinery to respond to, metabolize, and esterify cholesterol is found[6]. Thus, in response to exogenous cholesterol, tetrahymanol biosynthesis is curtailed and cholesterol (or its metabolites) is incorporated into the membrane in its place. Any cholesterol which is in

excess of membrane synthesis requirements is converted to cholesterol ester by an ACAT enzyme similar enough to the mammalian ACAT that it is inhibitable by Sandoz compound 58-035, a mammalian ACAT inhibitor, and is stored in cytoplasmic lipid droplets[6].

Many prokaryotes metabolize sterols to more water soluble products through hydroxylation reactions and by side chain cleavage, possibly to remove them from membranes. Interestingly, these are the mechanisms used by mitochondria in order to generate bile acids and steroid hormones. If the mitochondrion did arise through endosymbiosis as has been hypothesized[63], then it might have been forced to deal with eukaryotic sterols by these same reactions. The eukaryotic cell response could have been to utilize these more water-soluble steroids as hormones and emulsifying agents. It is equally interesting that ferns and some higher plants contain hopanoids and hopanoid-like compounds[76]. It is necessary to determine if the source of these compounds is the plant chloroplast, since this would also support an endosymbiotic origin. Cyanobacteria, from which chloroplasts are believed to have arisen, do in fact synthesize hopanoids[87].

Recently, a mutant of *E. coli* which is defective in the biosynthesis of negatively charged phospholipids was described[27]. At non-permissive temperatures, protein secretion was severely impaired in this mutant. A fascinating side effect of this response is the massive proliferation of intracellular membranes: mesosomes. This mutant therefore, might afford some important insight on how membrane lipid and protein synthesis/translocation are co-regulated during the generation of cellular membranes.

Summary

A number of studies of subcellular sterol distribution have indicated the relative richness in sterol of the plasma membrane although intracellular membranes are sterol poor. Interestingly, sterol synthesis proceeds in membranes of low sterol content. Thus, sterol must be moved from the site of synthesis to target membranes. While no mechanism has been firmly established, kinetics supportive of vesicular mediated transport have been obtained and sterol transfer proteins have been characterized. The translocation of proteins across the relatively uncondensed membrane of prokaryotes, coupled with the segregation of protein translocatory membranes to the cell interior away from the highly condensed plasma membrane in eukaryotes, suggests that membrane condensation caused by sterols may have been a driving force in the evolution of an intracellular membrane system.

Abbreviations used: ACAT, Acyl CoA:cholesterol acyltransferase; c/p cholesterol/phospholipid; ER, endoplasmic reticulum; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl Coenzyme A reductase; RER, rough endoplasmic reticulum, SCP, squalene and sterol carrier protein; SCP1 and SCP2, sterol carrier protein 1 and 2; SER smooth endoplasmic reticulum; SPF, supernatant protein factor.

1 Amar-Costesec, A., Wibo, M., Thines-Sempoux, D., Beaufay, H., and Berthet, J., Analytical study of microsomes and isolated subcellular membranes from rat liver IV. Biochemical, physical, and morphological modifications of microsomal components induced by digitonin, EDTA, and pyrophosphate. J. Cell Biol. *62* (1974) 717–745.
2 Appelkvist, E. L., In vitro labeling of peroxisomal cholesterol with radioactive precursors. Biosci. Rep. *7* (1987) 853–858.
3 Basu, J., Kundu, M., Bhattacharya, U., Mazumder, C., and Chakrabarti, P., Purification and characterisation of a nonspecific lipid transfer protein from goat liver. Biochim. biophys. Acta *959* (1988) 134–142.
4 Behnke, A., Tranum-Jensen, J., and Van Deurs, B., Filipin as a cholesterol probe. II. Filipin-cholesterol interaction in red blood cell membranes. Eur. J. Cell Biol. *35* (1984) 200–215.
5 Billheimer, J. T., and Gaylor, J. L., Cytosolic modulators of activities of microsomal enzymes of cholesterol biosynthesis. J. biol. Chem. *255* (1980) 8128–8135.
6 Billheimer, J. T., Landrey, J. R., and Conner, R. L., The presence of Acyl-CoA:cholesterol acyltransferase in *Tetrahymena pyriformis W.* Comp. Biochem. Physiol. *92B* (1989) 675–680.
7 Billheimer, J. T., Strehl, L. L., Davis, G. L., Strauss, J. F. III, and Davis, L. G., Characterization of a cDNA clone encoding rat sterol carrier protein 2. DNA, in press.
8 Bisseret, P., Wolff, G., Albrecht, A. M., Tanaka, T., Nakatani, Y., and Ourrison, G., A direct study of the cohesion of lecithin bilayers: the effect of hopanoids and α,w-dihydroxycarotenoids. Biochem. biophys. Res. Commun. *110* (1983) 320–324.
9 Blanchette-Mackie, E. J., Dwyer, N. K., Amende, L. M., Kruth, M. S., Butter, J. D., Sokol, J., Comly, M. E., Vanier, M. T., August, J. T., Brady, R. O., and Pentchev, P. G., Type-C Niemann-Pick disease: low density lipoprotein uptake is associated with premature cholesterol accumulation in the Golgi complex and excessive cholesterol storage in lysosomes. Proc. natl Acad. Sci. USA *85* (1988) 8022–8026.
10 Bloj, B., and Zilversmit, D. B., Rat liver proteins capable of transferring phosphatidylethanolamine. Purification and transfer activity for other phospholipids and cholesterol. J. biol. Chem. *252* (1977) 1613–1619.
11 Bloj, B., and Zilversmit, D. B., Complete exchangeability of cholesterol in phosphatidylcholine/cholesterol vesicles of different degrees of unsaturation. Biochemistry *16* (1977) 3943–3948.
12 Bloj, B., and Zilversmit, D. B., Accelerated transfer of neutral glycosphingolipids and ganglioside G by a purified lipid transfer protein. J. biol. Chem. *256* (1981) 5988–5991.
13 Bloom, M., and Mouritsen, O. G., The evolution of membranes. Can. J. Chem. *66* (1988) 706–712.
14 Blouin, A., Bolender, R. P., and Weibel, E. R., Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. J. Cell Biol. *72* (1977) 441–455.
15 Brasaemle, D. L., Robertson, A. D., and Attie, A. D., Transbilayer movement of cholesterol in the human erythrocyte membrane. J. Lipid. Res. *29* (1988) 481–489.
16 Brown, M. S., and Goldstein, J. L., A receptor-mediated pathway for cholesterol homeostasis. Science *232* (1986) 34–47.
17 Cavalier-Smith, T., in: Molecular and Cellular Aspects of Macrobial Evolution, pp. 33–84. Society for General Microbiology Ltd., Symposium 32. Eds M. J. Carlile, J. F. Collins and B. E. B. Moseley. Cambridge University Press 1981.
18 Chanderbhan, R., Noland, B. J., Scallen, T. J., and Vahouny, G. V., Sterol carrier protein 2, delivery of cholesterol from adrenal lipid droplets to mitochondria for pregnenolone synthesis. J. biol. Chem. *257* (1982) 8928–8934.
19 Chanderbhan, R., Tanaka, T., Strauss, J. F., Irwin, D., Noland, B. J., Scallen, T. J., and Vahouny, G. V., Evidence for sterol carrier protein 2-like activity in hepatic, adrenal and ovarian cytosol. Biochem. biophys. Res. Commun. *117* (1983) 702–709.
20 Chanderbhan, R. F., Kharroubi, A. T., Noland, B. J., Scallen, T. J., and Vahouny, G. V., Sterol carrier protein 2; Further evidence for its role in adrenal steroidogenesis. Endocr. Res. *12* (1986) 351–370.
21 Chesterton, C. J., Distribution of cholesterol precursors and other lipids among rat liver intracellular structures: evidence for the endoplasmic reticulum as the site of cholesterol and cholesterol ester synthesis. J. biol. Chem. *243* (1968) 1147–1151.
22 Chin, D. J., Luskey, K. L., Anderson, R. G. W., Faust, J. R., Goldstein, J. L., and Brown, M. S., Appearance of crystalloid endoplasmic reticulum in compactin-resistant Chinese hamster cells with a 500-fold increase in 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Proc. natl Acad. Sci. USA *79* (1982) 1185–1189.

23 Colbeau, A., Nachbaur, J., and Vignais, P. M., Enzymic characterization and lipid composition of rat liver subcellular membranes. Biochim. biophys. Acta *249* (1971) 462–492.

24 Coleman, R., and Finean, J. B., Preparation and properties of isolated plasma membranes from guinea pig tissues. Biochim. biophys. Acta *125* (1966) 197–206.

25 Comte, J., Maisterrena, B., and Gautheron, D. C., Lipid composition and protein profiles of outer and inner membranes from pig heart mitochondria-comparison with microsomes. Biochim. biophys. Acta *419* (1976) 271–284.

26 Crain, R. C., and Zilversmit, D. B., Two nonspecific phospholipid exchange proteins from beef liver. 1. Purification and characterization. Biochemistry *19* (1980) 1433–1439.

27 De Cock, H., Meeldijk, J., Overduin, P., Verkleij, A. J., and Tommassen, J., Membrane biogenesis in *Escherichia coli:* effects of a secA mutation. Biochim. biophys. Acta *985* (1989) 313–319.

28 DeGrella, R. F., and Simoni, R. D., Intracellular transport of cholesterol to the plasma membrane. J. biol. Chem. *257* (1982) 14 256–14 262.

29 Demel, R. A., Louwers, H., Jackson, R. L., and Wirtz, K. W. A., Protein-mediated lipid transfer between monolayers and bilayers. Coll. Surf. *10* (1984) 301–311.

30 Dempsey, M. E., Hargis, P. S., McGuire, D. M., McMahon, A., Olson, C. D., Salati, L. M., Clarke, S. D., and Towle, H. C., Role of sterol carrier protein in cholesterol metabolism. Chem. Phys. Lipids *38* (1985) 223–237.

31 DeSilva, N. S., and Siu, C.-H., Vesicle-mediated transfer of phospholipids to plasma membrane during cell aggregation of *Dictyostelium discoideum*. J. biol. Chem. *256* (1981) 5845–5850.

32 Engleman, D. M., and Steitz, T. A., The spontaneous insertion of proteins into and across membranes: the helical hairpin hypothesis. Cell *23* (1981) 411–422.

33 Ferguson, J. B., and Bloch, K., Purification and properties of a soluble protein activator of rat liver squalene epoxidase. J. biol. Chem. *252* (1977) 5381–5385.

34 Flesch, G., and Rohmer, M., Growth inhibition of hopane synthesizing bacteria by squalene cyclase inhibitors. Archs Microbiol. *147* (1987) 100–104.

35 Fuks-Holmberg, D., and Bloch, K., Intermembrane transfer of squalene promoted by supernatant protein factor. J. Lipid Res. *24* (1983) 402–408.

36 Gavey, K. L., and Scallen, T. J., Studies on the conversion of enzymatically generated microsome-bound squalene to sterol. J. biol. Chem. *253* (1978) 5476–5483.

37 Gordon, J. I., Alpers, D. H., Ockner, R. K., and Strauss, A. W., The nucleotide sequence of rat liver fatty acid binding protein mRNA. J. biol. Chem. *258* (1983) 3356–3363.

38 Gotlib, L. J., and Searls, D. B., Plasma membrane isolation on DEAE-sephadex beads. Biochim. biophys. Acta *602* (1980) 207–212.

39 Gottlieb, M. H., The reactivity of human erythrocyte membrane cholesterol with a cholesterol oxidase. Biochim. biophys. Acta *466* (1977) 422–428.

40 Green, C., The movement of cholesterol within cells, in: Sterol Biosynthesis and Function. Eds K. E. Suckling and B. C. Baldwin. Biochem. Soc. Trans. *11* (1983) 637–639.

41 Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitan, Z., Arias, I. M., and Jakoby, W. B., The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proc. natl Acad. Sci. USA *71* (1974) 3879–3882.

42 Hashimoto, S., Drevon, C. A., Weinstein, D. B., Bernett, J. S., Dayton, S., and Steinberg, D., Activity of acyl-CoA:cholesterol acyltransferase and 3-hydroxy-3-methylglutaryl-CoA reductase in subfractions of hepatic microsomes enriched with cholesterol. Biochim. biophys. Acta *754* (1983) 126–133.

43 Hodges, T. K., Leonard, R. T., Bracker, C. E., and Keenan, T. W., Purification of an ion stimulated adenosine triphosphatase from plant roots: association with plasma membranes. Proc. natl Acad. Sci. USA *69* (1972) 3307–3311.

44 Kandutsch, A. A., Chen, H. W., and Shown, E. P., Binding of 25-hydroxycholesterol and cholesterol to different cytosolic proteins. Proc. natl Acad. Sci. USA *74* (1977) 2500–2503.

45 Kandutsch, A. A., Chen, H. W., and Heiniger, H. J., Biological activity of some oxygenated sterols. Science *201* (1978) 498–501.

46 Kannenberg, E., Blume, A., McElhany, R. N., and Poralla, K., Monolayer and calorimetric studies of phosphatidylcholines containing branched chain fatty acids and their interactions with cholesterol and with a bacterial hopanoid in model membranes. Biochim. biophys. Acta *733* (1983) 111–116.

47 Kaplan, M. R., and Simoni, R. D., Transport of cholesterol from the endoplasmic reticulum to the plasma membrane. J. Cell Biol. *101* (1985) 446–453.

48 Keller, G. A., Barton, M. C., Shapiro, D. J., and Singer, S. S., 3-Hydroxy-3-methylglutaryl-coenzyme A reductase is present in peroxisomes in normal rat liver cells. Proc. natl Acad. Sci. USA *82* (1985) 770–774.

49 Keller, G. A., Pazirandeh, M., and Krisans, S., 3-Hydroxy-3-methylglutaryl coenzyme A reductase localization in rat liver peroxisomes and microsomes of control and cholestyramine-treated animals; quantitative biochemical and immunoelectron microscopical analyses. J. Cell Biol. *103* (1986) 875–886.

50 Keller, G. A., Scallen, T. J., Clarke, D., Maher, P. A., Krisans, S. K., and Singer, S. J., Sub-cellular localization of sterol carrier protein 2 in rat hepatocytes: its primary localization to peroxisomes. J. Cell Biol. *105* (1989) 1353–1361.

51 Kleinig, H., Nuclear membranes from mammalian liver II. Lipid composition. J. Cell Biol. *46* (1970) 396–402.

52 Krisans, S. K., Thompson, S. L., Pena, L. A., Kok, E., and Javitt, N. B., Bile acid synthesis by rat liver peroxisomes: metabolism of 26-hydroxycholesterol to 3-β-hydroxy-5-cholenoic acid. J. Lipid Res. *26* (1985) 1324–1332.

53 Lange, Y., Dolde, J., and Steck, T. L., The rate of transmembrane movement of cholesterol in the human erythrocyte. J. biol. Chem. *256* (1981) 5321–5323.

54 Lange, Y., and Matthies, H. J. G., Transfer of cholesterol from its site of synthesis to the plasma membrane. J. biol. Chem. *259* (1984) 14 624–14 630.

55 Lange, Y., and Muraski, M. F., Topographic heterogeneity in cholesterol biosynthesis. J. biol. Chem. *263* (1988) 9366–9373.

56 Lange, Y., and Ramos, B. V., Analysis of the distribution of cholesterol in the intact cell. J. biol. Chem. *258* (1983) 15 130–15 134.

57 Lange, Y., and Steck, T. L., Cholesterol-rich intracellular membranes: a precursor to the plasma membrane. J. biol. Chem. *260* (1985) 15 592–15 597.

58 Lange, Y., Swaisgood, M. H., Ramos, B. V., and Steck, T. L., Plasma membranes contain half of the phospholipid and 90 % of the cholesterol and sphingomyelin in cultured human fibroblasts. J. biol. Chem. *264* (1989) 3786–3793.

59 Li, A. C., Tanaka, R. D., Callaway, K., Fogelman, A. M., and Edwards, P. A., Localization of 3-hydroxy-3-methylglutaryl CoA reductase and 3-hydroxy-3-methylglutaryl CoA synthetase in the rat liver and intestine is effected by cholestyramine and mevinolin. J. Lipid Res. *29* (1988) 781–796.

60 Liscum, L., and Faust, J. R., Low density lipoprotein (LDL)-mediated suppression of cholesterol synthesis and LDL uptake is defective in Nieman-Pick type C fibroblasts. J. biol. Chem. *262* (1987) 17 002–17 608.

61 Loud, A. V., and Bucher, N. L. R., The turnover of squalene in relation to the biosynthesis of cholesterol. J. biol. Chem. *233* (1958) 37–41.

62 Luskey, K. L., Faust, J. R., Chin, D. J., Brown, M. S., and Goldstein, J. L., Amplification of the gene for 3-hydroxy-3-methylglutaryl coenzyme A reductase, but not for the 53 kDa protein, in UT-1 cells. J. biol. Chem. *258* (1983) 8462–8469.

63 Margulis, L., Origin of Eukaryotic Cells. Yale University Press, New Haven 1975.

64 McGookey, D. J., and Anderson, R. G. W., Morphological characterization of the cholesteryl ester cycle in cultured mouse macrophage foam cells. J. Cell Biol. *97* (1983) 1156–1168.

65 McLean, L. R., and Phillips, M. C., Mechanism of cholesterol and phosphatidylcholine exchange on transfer between unilamellar vesicles. Am. chem. Soc. *20* (1981) 2893–2900.

66 Mitropoulos, K. A., Venkatesan, S., Balasubramaniam, S., and Peters, T. J., The submicrosomal localization of 3-hydroxy-3-methylglutaryl coenzyme-A reductase, cholesterol 7a-hydroxylase and cholesterol in rat liver. Eur. J. Biochem. *82* (1978) 419–429.

67 Morris, H. R., Larsen, B. S., and Billheimer, J. T., A mass spectrometric study of the structure of sterol carrier protein SCP2 from rat liver. Biochem. biophys. Res. Commun. *154* (1988) 476–482.

68 Nesmeyanova, M. A., On the possible participation of acid phospholipids in the translocation of secreted proteins through the bacterial cytoplasmic membrane. FEBS Lett. *142* (1982) 189–193.

69 Noland, B. J., Arebalo, R. E., Hansbury, E., and Scallen, T. J., Purification and properties of sterol carrier protein 2. J. biol. Chem. *255* (1980) 4282–4289.

70 O'Malley, B. W., and Schrader, W. T., The receptors of steroid hormones. Sci. Am. *234* (1976) 32–43.

71 Ono, T., and Bloch, K., Solubilization and partial characterization of rat liver squalene epoxidase. J. biol. Chem. *250* (1975) 1571–1579.

72 Oparin, A. I., The Origin of Life (3rd English edition translated by S. Margulis). Macmillan, 1938.

73 Orci, L., Brown, M. S., Goldstein, J. L., Garcia-Segura, L. M., and Anderson, R. G. W., Increase in membrane cholesterol: a possible trigger for degradation of HMG-CoA reductase and crystalloid endoplasmic reticulum in UT-1 cells. Cell *36* (1984) 835–845.

74 Osborne, T. F., Goldstein, J. L., and Brown, M. S., 5' End of HMG-CoA reductase gene contains sequences responsible for cholesterol-mediated inhibition of transcription. Cell *42* (1985) 203–212.

75 Ourisson, G., Bigger and better hopanoids. Nature *326* (1987) 126–127.

76 Ourisson, G., Rohmer, M., and Poralla, K., Prokaryotic hopanoids and other polyterpenoid sterol surrogates. A. Rev. Microbiol. *41* (1987) 301–333.

77 Pastusyzn, A., Noland, B. J., Bazan, J. F., and Fletterick, R. J., Primary sequence and structural analysis of sterol carrier protein 2 from rat liver: homology with immunoglobulins. J. biol. Chem. *262* (1987) 13 219–13 227.

78 Pathak, R. K., Luskey, K. L., and Anderson, R. G. W., Biogenesis of the crystalloid endoplasmic reticulum in UT-1 cells: evidence that newly formed endoplasmic reticulum emerges from the nuclear envelope. J. Cell Biol. *102* (1986) 2158–2168.

79 Pentchev, R. G., Comly, M. E., Kruth, M. S., Tokoro, T., Butler, J., Sokol, J., Filling-Katz, M., Quirk, J. M., Marshall, D. C., Patel, S., Vanier, M. T., and Brady, R. O., Group C Niemann-Pick disease: faulty regulation of low-density lipoprotein uptake and cholesterol storage in cultured fibroblasts. FASEB J. *1* (1987) 40–45.

80 Pfleger, R. C., Anderson, N. G., and Snyder, F., Lipid class and fatty acid composition of rat liver plasma membranes isolated by zonal centrifugation. Biochemistry *7* (1968) 2826–2833.

81 Phillips, M. C., Johnson, W. J., and Rothblat, G. H., Mechanisms and consequences of cellular cholesterol exchange and transfer. Biochim. biophys. Acta *906* (1987) 223–276.

82 Prince, R. C., Hopanoids: the world's most abundant molecules? TIBS *12* (1987) 455–456.

83 Randall, L. L., Hardy, S. J. S., and Thom, J. R., Export of protein: A biochemical view. A. Rev. Microbiol. *41* (1987) 507–541.

84 Reinhart, M. P., Billheimer, J. T., Faust, J. R., and Gaylor, J. L., Subcellular localization of the enzymes of cholesterol biosynthesis and metabolism in rat liver. J. biol. Chem. *262* (1987) 9649–9655.

85 Reinhart, M. P., Billheimer, J. T., and Usher, D., Cytolipophorins: lipoprotein-like particles in the cytoplasm of phylogenetically diverse eukaryotes. J. Cell Biol. *107* (1988) 654a, abstr. no. 3715.

86 Ritter, M. C., and Dempsey, M. E., Specificity and role in cholesterol biosynthesis of a squalene and sterol carrier protein. J. biol. Chem. *246* (1971) 1536–1547.

87 Rohmer, M., Bouvier-Nave, P., and Ourisson, G., Distribution of hopanoid triterpenes in prokaryotes. J. gen. Microbiol. *130* (1984) 1137–1150.

88 Saat, Y. A., and Bloch, K. E., Effect of a supernatant protein on microsomal squalene epoxidase and 2,3-oxidosqualene-lanosterol cyclase. J. biol. Chem. *251* (1976) 5155–5160.

89 Scallen, T. J., Noland, B. J., Gavey, K. L., Bass, N. M., Ockner, R. K., Chanderbhan, R., and Vahouny, G. V., Sterol carrier protein 2 and fatty acid-binding protein. J. biol. Chem. *260* (1985) 4733–4739.

90 Scallen, T. J., Pastuszyn, A., Noland, B. J., Chanderbhan, R., Kharroubi, A., and Vahouny, G. V., Sterol carrier and lipid transfer proteins. Chem. Phys. Lipids *38* (1985) 239–261.

91 Scallen, T. J., Seetharam, B., Srikantaiah, M. V., Hansbury, E., and Lewis, M. K., Sterol carrier protein hypothesis: requirement for three substrate-specific soluble proteins in liver cholesterol biosynthesis. Life Sci. *16* (1975) 853–874.

92 Scallen, T. J., Srikantaiah, M. V., Seetharam, B., Hansbury, E., and Gavey, K. L., Sterol carrier protein hypothesis. Fedn Proc. *33* (1974) 1733–1746.

93 Scallen, T. J., Schuster, M. W., Dhar, A. K., and Skirdlant, H. B., Enzymic synthesis of cholesterol: use of a liver acetone powder. Lipids *6* (1971) 162–164.

94 Schroeder, F., Perlmutter, J. F., Glaser, M., and Vagelos, P. R., Isolation and characterization of subcellular membranes with altered phospholipid composition from cultured fibroblasts. J. biol. Chem. *251* (1976) 5015–5026.

95 Sinensky, M., and Strobel, G., Chemical composition of cellular fractions enriched in plasma membranes from sugar cane. Plant Sci. Lett. *6* (1976) 209–214.

96 Singer, I. I., Scott, S., Kazazis, D. M., and Huff, J. W., Lovastatin, an inhibitor of cholesterol synthesis, induces hydroxymethylglutaryl-coenzyme A reductase directly on membranes of expanded smooth endoplasmic reticulum in rat hepatocytes. Proc. natl Acad. Sci. USA *85* (1988) 5264–5268.

97 Singer, S. J., Maher, P. A., and Yaffe, M. P., On the translocation of proteins across membranes. Proc. natl Acad. Sci. USA *84* (1987) 1015–1019.

98 Srikantaiah, M. V., Hansbury, E., Loughran, E. D., and Scallen, T. J., Purification and properties of sterol carrier protein 1. J. biol. Chem. *251* (1976) 5496–5504.

99 Sziegoleit, A., Purification and characterization of a cholesterol-binding protein from human pancreas. Biochem. J. *207* (1982) 573–582.

100 Sziegoleit, A., A novel proteinase from human pancreas. Biochem. J. *219* (1984) 735–742.

101 Tai, M.-H., and Bloch, K., Squalene epoxidase of rat liver. J. biol. Chem. *247* (1972) 3767–3773.

102 Tanaka, T., Billheimer, J. T., and Strauss, J. F., Luteinized rat ovaries contain a sterol carrier protein. Endocrinology *114* (1984) 533–540.

103 Taylor, F. R., Saucier, S. E., Shown, E. P., Parish, E. J., and Kandutsch, A. A., Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of hydroxymethylglutaryl coenzyme A reductase. J. biol. Chem. *259* (1984) 12 382–12 387.

104 Taylor, F. R., Kandutsch, A. A., Gayen, A. K., Nelson, J. A., Nelson, S. S., Phirwa, S., and Spencer, T. A., 24,25-Epoxysterol metabolism in cultured mammalian cells and repression of 3-hydroxy-3-methylglutaryl-CoA reductase. J. biol. Chem. *261* (1986) 15 039–15 044.

105 Taylor, F. R., and Kandutsch, A. A., in: The Oxysterol Receptor in Sterol/Steroid Hormone Mechanism of Action, pp. 395–407. Eds R. Kumar, and T. Spelsberg. Martinus Nijhoff, Boston 1987.

106 Tchen, T. T., and Bloch, K., On the conversion of squalene to lanosterol in vitro. J. biol. Chem. *226* (1957) 921–930.

107 Teerlink, T., Van Der Krift, T. P., Van Heusden, G. P., and Wirtz, K. W. A., Determination of nonspecific lipid transfer protein in rat tissues and Morris hepatomas by enzyme immunoassay. Biochim. biophys. Acta *793* (1984) 251–259.

108 Thines-Sempoux, D., Amar-Costesec, A., Beaufay, H., and Berthet, J., The association of cholesterol, 5'-nucleotidase and alkaline phosphodiesterase I with a dinstinct group of microsomal particles. J. Cell Biol. *43* (1969) 189–192.

109 Thompson, S. L., Burrows, R., Laub, R. J., and Krisans, S. K., Cholesterol synthesis in rat liver peroxisomes. Conversion of mevalonic acid to cholesterol. J. biol. Chem. *262* (1987) 17 420–17 425.

110 Trzaskos, J. M., and Gaylor, J. L., Cytosolic modulators of activities of microsomal enzymes of cholesterol biosynthesis. Purification and characterization of a non-specific lipid-transfer protein. Biochim. biophys. Acta *751* (1983) 52–65.

111 Trzeciak, W. H., Simpson, E. R., Scallen, T. J., Vahouny, G. V., and Waterman, M. R., Studies on the synthesis of sterol carrier protein 2 in rat adrenocortical cells in monolayer culture. J. biol. Chem. *262* (1987) 3713–3717.

112 Tsuneoka, M., Yamamoto, A., Fujiki, Y., and Tashiro, Y., Non-specific lipid transfer protein (sterol carrier protein 2) is located in rat liver peroxisomes. J. Biochem. *104* (1988) 560–564.

113 Ulsamer, A. G., Wright, P. L., Wetzel, M. G., and Korn, E. D., Plasma and phagosome membranes of *Acanthamoeba castellanii*. J. Cell Biol. *51* (1971) 193–215.

114 Vahouny, G. V., Chanderbhan, R., Noland, B. J., Irwin, D., Dennis, P., Lambeth, J. D., and Scallen, T. J., Sterol carrier protein 2. J. biol. Chem. *258* (1983) 11 731–11 737.

115 Van Amerongen, A., Teerlink, T., Van Heusden, G. P. H., and Wirtz, K. W. A., The non-specific lipid transfer protein (sterol carrier protein 2) from rat and bovine liver. Chem. Phys. Lipids *38* (1985) 195–204.

116 Van Amerongen, A., Helms, J. B., van der Krift, T. P., Schutgens, R. B. H., and Wirtz, K. W. A., Purification of nonspecific lipid transfer protein (sterol carrier protein 2) from human liver and its deficiency in livers from patients with cerebro-hepato-renal (Zellweger) syndrome. Biochim. biophys. Acta *919* (1987) 149–155.

117 Van Deenen, L. L. M., and DeGier, J., Lipids of the red cell membrane, in: The Red Blood Cell, 2nd edn, pp. 147–211. Ed. D. M. Surgenor. Academic Press, New York 1974.

118 Van der Krift, T. P., Leunissen, J., Teerlink, T., Van Heusden, G. P. H., Verkleij, A. J., and Wirtz, K. W. A., Ultrastructural localization of a peroxisomal protein in rat liver using the specific anti-

body against the non-specific lipid transfer protein (sterol carrier protein 2). Biochim. biophys. Acta *812* (1985) 387–392.
119 Van Meer, G., Plasma membrane cholesterol pools. TIBS *12* (1987) 375–376.
120 Verner, K., and Schatz, G., Protein translocation across membranes. Science *241* (1988) 1307–1313.
121 Von Heijne, G., and Blomberg, C., Trans-membrane translocation of proteins. The direct transfer model. Eur. J. Biochem. *97* (1979) 175–181.
122 Wattenberg, B. W., and Silbert, D. F., Sterol partitioning among intracellular membranes-testing a model for cellular sterol distribution. J. biol. Chem. *258* (1983) 2284–2289.
123 Westerman, J., and Wirtz, K. W. A., The primary structure of the nonspecific lipid transfer protein. Biochem. biophys. Res. Commun. *127* (1985) 333–338.
124 Wickner, W. T., and Lodish, H. F., Multiple mechanisms of protein insertion into and across membranes. Science *230* (1985) 400–407.
125 Wieland, F. T., Gleason, M. L., Serafini, T. A., and Rothman, J. E., The rate of bulk flow from the endoplasmic reticulum to the cell surface. Cell. *50* (1987) 289–300.
126 Yeagle, P. L., Cholesterol and the cell membrane. Biochim. biophys. Acta *822* (1985) 267–287.

0014-4754/90/060599-13$1.50 + 0.20/0

Glycolipid transfer protein and intracellular traffic of glucosylceramide

T. Sasaki

Department of Biochemistry, Cancer Research Institute, Sapporo Medical College, South-1, West-17, Sapporo 060 (Japan)

Summary. Glycolipid transfer protein (GL-TP), a nonglycosylated protein with a molecular weight of 22,000 K, has been purified from pig brain. The protein transfers, by a carrier mechanism, glycolipids with a β-glucosyl or β-galactosyl residue directly linked to either ceramide or diacylglycerol. GL-TP appears to be present in most animal cells, and evidence has been obtained which indicates that it is a cytoplasmic protein. Little is known about the function of GL-TP. Current evidence indicates that glycosphingolipid glycosylation occurs at the luminal side of the Golgi apparatus, except for the glucosylation of ceramide, which has been shown to occur at the cytoplasmic side of the Golgi or endoplasmic membrane. It appears most likely that GL-TP participates in the intracellular traffic of glucosylceramide.
Key words. Glycosphingolipid; topography of glycolipid glycosylation; the Golgi apparatus; glucosylceramide; monensin; glycolipid transfer protein.

Intracellular location of glycosphingolipids and of enzymes of glycosphingolipid biosynthesis

Glycosphingolipids are localized predominantly, if not exclusively, in the outer leaflet of the plasma membrane[31]. It is now known that the glycosylation of glycosphingolipids occurs by the sequential addition of monosaccharides from sugar nucleotides to an acceptor, and is catalyzed by glycosyltransferases located in the Golgi membrane[20, 25, 35, 37, 42, 50]. Although the localization of glycolipid glycosyltransferases in specific cisternae in discrete parts of the Golgi apparatus has not been investigated experimentally one might expect, by analogy with glycoprotein glycosyltransferases[18, 38], that such a localization does occur. The sequential glycosylation of one glycosphingolipid to the next higher homologue involves a very small pool of intermediates which does not mix with the main pool of cellular glycosphingolipids[31]. Current evidence indicates that the small pool of intermediates is located in the Golgi apparatus. It is assumed that the endoplasmic reticulum is the site of ceramide biosynthesis, since fatty acid incorporation generally occurs in the endoplasmic reticulum.

Transport of glycosphingolipids from the Golgi apparatus to the plasma membranes

Very little is known about the mechanism of glycosphingolipid transport from the site of synthesis to the plasma membrane. Dower et al.[17] examined the kinetics of ganglioside transport from an intracellular site of synthesis to the plasma membrane in cultured cells. These authors distinguished surface and intracellular gangliosides by oxidizing cell surface gangliosides with sodium periodate and reacting the oxidized gangliosides with dinitrophenylhydrazine. It was found that the transfer of gangliosides from the site of synthesis to the cell surface required approximately 20 min. A variety of drugs, including inhibitors of protein synthesis and energy metabolism, modulators of cytoskeleton, and monensin, had no effect on the transport of newly synthesized GD_{1a} ganglioside to the plasma membrane[32]. Only low temperature effectively blocked the translocation. It appears that vesicular transport[21, 33] is the most likely mechanism for the transport of glycosphingolipids from the site of synthesis to the plasma membrane[32]. According to the vesicular transport model, glycosphingolipids are trans-