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Lipid transport pathways in mammalian cells

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Summary. A major deficit in our understanding of membrane biogenesis in eukaryotes is the definition of mechanisms by which the lipid constituents of cell membranes are transported from their sites of intracellular synthesis to the multiplicity of membranes that constitute a typical cell. A variety of approaches have been used to examine the transport of lipids to different organelles. In many cases the development of new methods has been necessary to study the problem. These methods include cytological examination of cells labeled with fluorescent lipid analogs, improved methods of subcellular fractionation, in situ enzymology that demonstrates lipid translocation by changes in lipid structure, and cell-free reconstitution with isolated organelles. Several general patterns of lipid transport have emerged but there does not appear to be a unifying mechanism by which lipids move among different organelles. Significant evidence now exists for vesicular and metabolic energy-dependent mechanisms as well as mechanisms that are clearly independent of cellular ATP content.
Key words. Lipids; membranes; transport; organelles; vesicles.

The major structural components of cell membranes are phospholipids, sphingolipids and cholesterol. Analysis of the total lipid content of an average nucleated mammalian cell reveals a composition of 45–55% phosphatidylcholine, 15–25% phosphatidylethanolamine, 10–15% phosphatidylinositol, 5–10% phosphatidyl-

serine, 1–2% phosphatidic acid, 5–10% sphingomyelin, 2–5% biphosphatidylglycerol (cardiolipin), 2–5% glycosphingolipids and 10–20% cholesterol[14]. The distribution of these lipids is inhomogeneous among the organelles of the cell[14,20,60].

The most striking differences in lipid composition between organelles are found when comparing the plasma membrane with the mitochondrial membranes[5]. Both sphingomyelin and cholesterol are markedly enriched in the plasma membrane while virtually absent from the mitochondria. Glycosphingolipids are mostly concentrated in the plasma membrane while being virtually absent from mitochondria and the endoplasmic reticulum[44,57]. The mitochondria are also very low in phosphatidylserine content while being the exclusive site of bisphosphatidylglycerol. The differences in composition of the major lipid classes among other subcellular membranes are less striking. There is a gradual enrichment of cholesterol in membranes as one moves along the path of endoplasmic reticulum → cis Golgi → trans Golgi → plasma membrane[8,33,57]. This observation is also true for sphingomyelin[57]. Phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol are distributed among all membranes but the levels may vary significantly among different membranes[5,13,20,60]. In addition to the heterogeneous distribution of lipid classes among membranes there is also an asymmetric distribution of lipids across the transverse axis of certain cell membranes[32,59]. This asymmetry is especially evident in the plasma membranes of cells in which sphingomyelin and glycosphingolipids are found almost exclusively in the external facing leaflet and phosphatidylserine and phosphatidylethanolamine are localized to the internal leaflet. The sequestration of phosphatidylserine at the inner leaflet of the plasma membrane of red cells and platelets has been shown to be functionally important[2,42]. The observed lack of homogeneity of lipids among membranes indicates that processes must exist which establish and maintain the non-random distribution of these molecules. These observations implicate specific transport processes for lipids in organelle biogenesis.

These processes are of two types, 1) *interorganelle transport,* which is involved in moving lipids from the site of synthesis (principally the endoplasmic reticulum but also including Golgi and nuclear membranes)[3,16,54,56] to organelles incapable of synthesizing some or all of the lipids de novo such as plasma, mitochondrial and lysosomal membranes;

2) *intraorganelle transport* which includes the transbilayer movement of lipid in the endoplasmic reticulum and plasma membrane and the movement of lipids within and between the inner and outer mitochondrial membranes. This chapter will primarily emphasize interorganelle translocation and selected aspects of intraorganelle movement. A schematic summary of the lipid transport processes described in this chapter is given in the figure.

More detailed information on additional aspects of intraorganelle transport phenomena are given in this volume in the article by Devaux and Zachowski[12]. The reader is also referred to other recent reviews on lipid transport[3,8,45,57,60].

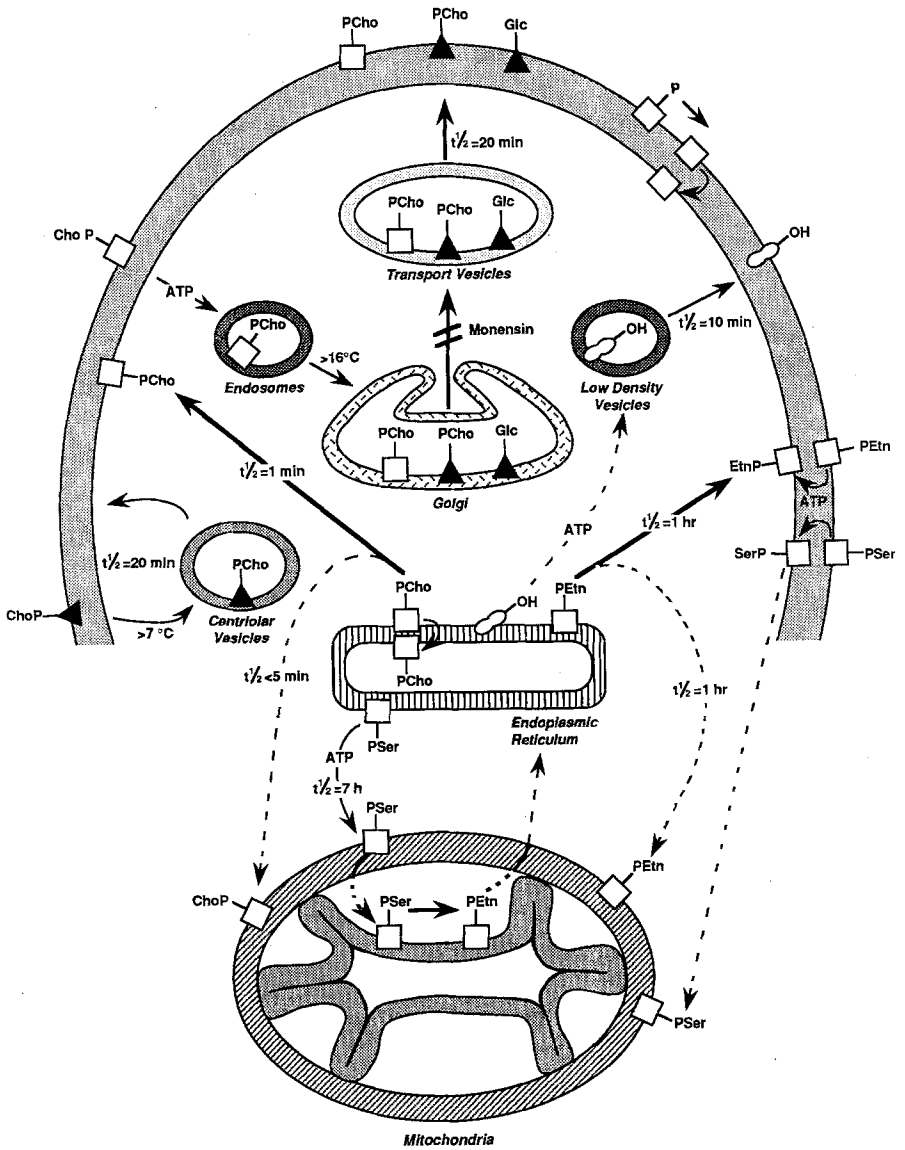
A detailed discussion of phospholipid exchange/transfer proteins has not been included in this chapter. A large body of evidence demonstrates that these proteins are capable of effecting lipid transfer among membrane populations in cell-free systems. The lipids transferred include phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, bisphosphatidylglycerol, sphingomyelin, cholesterol and some glycosphingolipids. In most, but not all cases, the transfer observed is an exchange of lipid species between donor and acceptor membranes rather than a net transfer of mass. A review of the properties of these proteins is included in the article by Wirtz and Gadella in this volume[66].

Lipid transport to the plasma membrane

Phosphatidylcholine

Phosphatidylcholine constitutes approximately 25 mole percent of total lipids in the plasma membrane. The principal site of synthesis of this lipid is the endoplasmic reticulum and significant synthetic activity has also been found in the Golgi membranes[3,16,54,56]. Kaplan and Simoni have utilized rapid plasma membrane isolation methods for CHO-K1 cells to examine the arrival of pulse labeled [^{3}H]-phosphatidylcholine at this membrane[17]. Newly synthesized phosphatidylcholine was found to arrive at the plasma membrane with a t½ of approximately 1 min. The translocation process was independent of cellular ATP levels and occurred at 15 °C. The inhibitors colchicine, monensin and CCCP also failed to disrupt phosphatidylcholine transport. In addition to the rapid transport component, evidence was presented for a second component that reached the plasma membrane over a 2-h period. The rapid phosphatidylcholine transport accounted for 90% of this lipid delivered to the plasma membrane. This rapid appearance of phosphatidylcholine at the cell surface occurs at rates much faster than protein movement from the endoplasmic reticulum to the plasma membrane. Thus by both kinetic and energetic criteria the majority of phosphatidylcholine transport from the endoplasmic reticulum appears to be independent of vesicle traffic that characterizes protein delivery to the surface membrane. The mechanism by which this rapid movement of lipid occurs has not yet been elucidated but the data are consistent with an energy-independent soluble carrier such as the phospholipid exchange proteins originally described by Wirtz and Zilversmit[67] and discussed in detail in the article by Wirtz and Gadella in this volume[66].

Another method of measuring phosphatidylcholine assembly into the plasma membrane has employed the flu-



Schematic summary of lipid translocations in animal cells. Major lipid translocation processes in this chapter are shown:
□, the diacylglycerol portion of phospholipids; ▲, the ceramide portion of sphingolipids.  is the symbol for cholesterol. Abbreviations: Cho, choline; Etn, ethanolamine; Ser, serine; Glc, glucose. The dotted lines indicate that the translocation processes have been shown to occur but studies of mechanisms have not been conducted. The placement of the lipid in a given leaflet of the membrane bilayer is deliberate and indicates experimental evidence for the location.

orescent analog of phosphatidylcholine, NBD-phosphatidylcholine (1-acyl, 2-(N-4-nitro-benzo-2 oxa-1,3 diazole) amino caproyl phosphatidylcholine). Using this fluorescent lipid Sleight and Pagano[48] were able to selectively label a Golgi-endosomal pool with NBD-phosphatidylcholine. This pool was originally derived from the plasma membrane and most probably represents a pathway by which phosphatidylcholine is recycled to and from the plasma membrane. The movement of the fluorescent lipid within the cell was monitored by fluorescence microscopy and endogenous phospholipase degradation. The fluorescence present in the Golgi apparatus diminished with time at 37 °C and the loss of fluorescence was attributable to phospholipase degradation. Additional data from these authors indicate that a plasma membrane phospholipase would account for significant rates of degradation of the NBD-phosphatidylcholine. Although the data are subject to several interpretations, Sleight and Pagano[48] favor vesicle-based delivery of fluorescent lipid to the cell surface followed by degradation.

Thus, experiments investigating phosphatidylcholine movement to the plasma membrane suggest that this transport can occur by at least two different processes; an ATP-independent mechanism that is coupled to biosynthesis, and an ATP-dependent mechanism most likely involving transport vesicles which cycle among the plasma membrane, endosomal vesicles and Golgi apparatus compartments.

Phosphatidylethanolamine

The translocation of phosphatidylethanolamine to the plasma membrane of Chinese hamster lung fibroblasts was examined by Sleight and Pagano[47] using a [^{3}H] ethanolamine labeling followed by modification of the outer leaflet of the plasma membrane of the cells with trinitrobenzene sulfonate (TNBS). The reaction of TNBS with the cell surface phosphatidylethanolamine forms trinitrophenyl phosphatidylethanolamine which is readily separable from the underivatized lipid and therefore the pool of phosphatidylethanolamine that faces the external environment can be distinguished from all other pools of phosphatidylethanolamine. Thus, this method measures the overall transport rate between the site of synthesis of phosphatidylethanolamine (from ^{3}H ethanolamine) and the outer leaflet of the plasma membrane. The results of these experiments demonstrate that phosphatidylethanolamine appears at the plasma membrane very rapidly after synthesis without a discernible lag time. Although the initial appearance of labeled phosphatidylethanolamine is rapid, the amount of labeled phosphatidylethanolamine arriving at the plasma membrane (as judged by TNBS modification) continues to increase for 2 h. Likewise, pulse-chase experiments demonstrate that radioequilibration between the intracellular and TNBS accessible pool requires approximately 2 h. The transport processes are insensitive to the metabolic poisons monensin, colchicine, cytocholasins B and D, nocodazole, and deoxyglucose plus azide, and occur at rates faster than those of newly synthesized proteins. In addition, the movement of this lipid is not blocked by reduced temperatures that are capable of disrupting vesicle traffic. The transport of the newly synthesized phosphatidylethanolamine has also been examined in mitotic cells which have arrested vesicular transport[21]. The appearance of nascent phosphatidylethanolamine at the plasma membrane is similar for mitotic and interphase cells. These results make vesicular transport of phosphatidylethanolamine to the plasma membrane unlikely, and are consistent with the hypothesis that soluble carriers, such as lipid transfer proteins function to deliver this lipid to the cell surface membrane.

Sphingolipids

Sphingolipids are markedly enriched in the outer leaflet of the plasma membrane of animal cells[59]. The concentration of glycosphingolipids and sphingomyelin in the apical portion of polarized epithelial cells is especially striking[44,58]. The transport of sphingomyelin and simple glycosphingolipids such as glucocerebroside to the plasma membrane of fibroblasts has been examined by fluorescence microscopy after labeling cells with NBD-ceramide[27,28]. When fibroblasts are labeled with NBD-ceramide at 2 °C there is a partitioning of this lipid among all of the cell membranes. However, when the cells are subsequently warmed to 37 °C the NBD-ceramide concentrates in the Golgi apparatus and is converted to glucosyl-NBD-ceramide and NBD-sphingomyelin. These mature sphingolipids are subsequently transported from the Golgi to the outer leaflet of the cell surface bilayer with a t½ of 20–30 min. The kinetics of transport are similar to those found for plasma membrane proteins. In addition, the transport is reversibly blocked by the ionophore monensin[28] which suggests a vesicular transport mechanism. This process has also been investigated in mitotic cells where vesicle trafficking is interrupted[21]. In mitotic cells the metabolism of NBD-ceramide to glucosyl-NBD-ceramide and NBD-sphingomyelin was similar to interphase cells but transport to the cell surface was blocked.

A further refinement of this transport process in epithelial cells has been investigated by van Meer et al.[58]. The epithelial cells transport NBD-sphingomyelin and NBD glucosylceramide to their cell surface with kinetics similar to those observed in fibroblasts. However, the apical plasma membrane is relatively enriched in glucosylceramide and the delivery of NBD-glucosylceramide to this membrane was 2–3-fold greater than for the basolateral membrane. These results suggest that selective apical sorting of NBD-glucosylceramide occurs at the level of the trans Golgi[44,57].

Cholesterol

Cholesterol is a major lipid component of the plasma membrane constituting 30–40 mol percent of the total lipid present[14,26,60]. The enzymes of cholesterol biosynthesis are located in microsomal fractions derived from rat liver[39], however, in fibroblasts the location of the numerous synthetic enzymes appears more heterogenous[25]. The arrival of newly synthesized cholesterol at the plasma membrane has been examined in pulse chase experiments that use either selective rapid isolation of plasma membrane[9,18] or chemical modification of outer leaflet cholesterol using cholesterol oxidase[24]. With plasma membrane isolation methods the t½ for cholesterol transport to the surface membrane is 10 min. The cholesterol oxidase method gives a t½ of 1–2 h. The discrepancy in t½ values has not yet been resolved although the cholesterol oxidase method only measures cholesterol within the outer leaflet of the plasma membrane and this may be a critical difference to consider when interpreting the results. The delivery of cholesterol to the plasma membrane was shown to be reversibly inhibited by the depletion of cellular ATP but insensitive to cytocholasin B, monensin and colchicine[9,18]. Transport of cholesterol was arrested at 15 °C and subcellular fractionation indicated that this lipid may accumulate in a unique low density fraction[18,24]. The kinetic, inhibitor, low temperature, and centrifugation results are suggestive of a vesicular transport process. The inability of monensin to affect this process suggests that the transport intermediate at some step must be distinct from that followed by NBD-sphingomyelin and NBD-glucosylceramide in their transport to the plasma membrane. A more detailed

discussion of intracellular sterol transport appears in this issue in the article by Reinhart[38].

Lipid translocation from the plasma membrane

A number of studies have provided clear evidence of lipid dynamics at the plasma membrane that demonstrate transbilayer movement of lipid and the export of lipids from this membrane to other organelles. In some instances this transport reflects constitutive recycling of membrane to endosomes and the Golgi apparatus; but there also exists clear examples of the net transport of lipids from the plasma membranes to other organelles.

Phosphatidylcholine

One method of examining phosphatidylcholine transport processes associated with the plasma membrane employs NBD-phosphatidylcholine[48]. The monomeric solubility of this lipid coupled with its high partition coefficient for hydrophobic environments affords investigators the opportunity to efficiently transplant this lipid into the outer leaflet of the plasma membrane. Sleight and Pagano[48] demonstrated that Chinese hamster V79 fibroblasts readily take up NBD-phosphatidylcholine at 2 °C. Maintenance of the cells at 2 °C results in the reversible and selective labeling of the plasma membrane. When these cells are subsequently warmed to 37 °C up to 50 % of the fluorescent label is degraded within 30 min and lost from the cell. The remainder of the fluorescent lipid is located at either the plasma membrane or in intracellular structures that by independent criteria are identifiable as elements of the Golgi apparatus. The movement of NBD-phosphatidylcholine to the Golgi is blocked by depleting cellular ATP levels with sodium azide and deoxyglucose. In addition, incubation of the cells at 16 °C leads to fluorescent labeling of another cell compartment most likely endosomes that accumulate because they are unable to fuse with the Golgi apparatus. The phosphatidylcholine pool observed by these methods resides in a spatially segregated domain that corresponds to the outer leaflet of the plasma membrane, the inner (non-cytoplasmic) leaflet of endosomal membranes and the inner (non-cytoplasmic) leaflet of Golgi. The data suggest that phosphatidylcholine in these specific compartments is essentially trapped and cannot undergo significant rates of transbilayer movement to the opposite leaflet of the membrane bilayers. The theoretical basis for this latter conclusion is the known partitioning properties of NBD-phosphatidylcholine (were the lipid to gain access to cytoplasmic facing leaflets of the bilayer this would lead to the nonspecific partitioning of the lipid among all cytoplasmic membrane domains). The empirical basis for this conclusion derives from microinjection[36] experiments in which liposomes containing NBD-phosphatidylcholine that are introduced into the cell cytoplasm cause the nonspecific labeling of all cell membranes. Independent data examining the transbilayer movement of radiolabeled phosphatidylcholine in phagosomal-endosomal membranes corroborates these conclusions[41].

The movement of NBD-phosphatidylcholine from the plasma membrane to intracellular structures may not follow the same route in all cells. Recent work by Sleight and Abanto[46] demonstrates that the internalization of the fluorescent lipid may fail to occur in Vero cells and may be targeted to structures other than the Golgi apparatus in BHK, CHO-15B MDCK and WI-38 cells. The data further demonstrate that in SV-40 transformed WI-38 cells the barrier to transbilayer movement of phosphatidylcholine at the plasma membrane has been lost. Exogenous phosphatidylcholine has also been shown to interact with cells in functionally important ways using somatic cell mutants defective in phosphatidylcholine synthesis[13]. These cells which exhibit thermolabile CDP-choline synthetase and are temperature sensitive for growth can bypass the mutation upon supplementation of cultures with liposomal forms of phosphatidylcholine and several analogs including D-phosphatidylcholine and beta-phosphatidylcholine (1,3 diacyl *sn* glycero-2-phosphocholine). Although the mechanism by which these phosphatidylcholines substitute for the normal cellular complement of lipid are unknown, the results clearly demonstrate that cells possess routes for assimilating and redistributing exogenous phosphatidylcholine, and the first step must involve interactions at the plasma membrane.

Phosphatidylethanolamine

The fluorescent lipid analog NBD-phosphatidylethanolamine readily inserts into the outer leaflet of the plasma membrane of Chinese hamster fibroblasts at 2 °C[49]. When such labeled cultures are shifted to 37 °C, there is prominent labeling of the nuclear envelope, mitochondria and the Golgi apparatus. The labeling of the Golgi apparatus but not the other membranes can be blocked by shifting the labeled cells to 7 °C. Modest depletion of cellular ATP levels (to approximately 80 % of control cells) prevents the endocytosis and labeling of the Golgi apparatus but not the appearance of fluorescence in the mitochondria and nuclear envelope. These results are consistent with NBD-phosphatidylethanolamine being disseminated throughout the cell by both endocytic pathways (as also seen for NBD-phosphatidylcholine), and non-endocytic partitioning pathways that are properties of the fluorescent lipid per se and not necessarily properties of native phosphatidylethanolamine.

More severe ATP depletion (to approximately 20 % of unpoisoned cells) of the cells concomitant with a shift from 2 °C to 37 °C blocks the internalization of NBD-phosphatidylethanolamine[29]. These results have been interpreted to mean that the internalization of NBD-phosphatidylethanolamine is a multi-step process consisting of 1) insertion of exogenous lipid into the outer leaflet of the plasma membrane, 2) transbilayer movement of lipid from the outer leaflet to the inner leaflet of the plasma

membrane, (this process requires ATP and occurs at 7 °C but not 2 °C), 3) nonspecific partitioning of NBD-phosphatidylethanolamine from the inner leaflet of the plasma membrane to other organelles, and 4) endocytosis of NBD-phosphatidylethanolamine along with bulk phase endocytosis of the plasma membrane, (this process requires ATP and does not occur at 7 °C), 5) fusion of endosomes with the Golgi apparatus.

Evidence that the transbilayer movement of phosphatidylethanolamine occurs as well as the NBD-analog has been presented by other investigators using the erythrocyte to study the process. Siegneuret and Devaux demonstrated that spin-labeled analogs of phosphatidylethanolamine underwent transbilayer movement with a t½ of 1 h at 37 °C and this process required ATP[43]. Tilley et al.[53] using radiolabeled phospholipids inserted into the plasma membrane of erythrocytes by the action of phospholipid exchange proteins demonstrated (by accessibility to exogenous phospholipases) that phosphatidylethanolamine moved from the outer leaflet to the inner leaflet of the bilayer with t½ of approximately 1 h. These investigators also presented evidence that the transbilayer migration of the lipid required ATP. Similar conclusions regarding an ATP requirement for the transbilayer movement of phosphatidylethanolamine were reached by Daleke and Huestis[7] using cell morphology as an indicator of hemileaflet phosphatidylethanolamine content. In addition to requiring ATP the translocator activity appears to require free sulfhydryl moieties as its activity is inhibited by N-ethylmaleimide, iodoacetamide and diamide.

Phosphatidylserine

Phosphatidylserine is a minor component of the plasma membrane and it has been localized to the inner (cytoplasmic) side of the bilayer[59]. Studies investigating the interaction of NBD-phosphatidylserine with Chinese hamster fibroblasts reveal translocation phenomena similar to that observed using NBD-phosphatidylethanolamine[29,49]. Subsequent to the partitioning of this fluorescent lipid into the outer leaflet of the plasma membrane of fibroblast at 2 °C it undergoes the following translocations: 1) at 7 °C the NBD-phosphatidylserine moves from the outer leaflet to the inner leaflet of the plasma membrane, 2) from the inner leaflet, the fluorescent lipid nonspecifically partitions among intracellular membranes. The transbilayer movement of NBD-phosphatidylserine in the first step is inhibited by depletion of ATP to 20 % of the levels found in control cells. Glutaraldehyde and N-ethylmaleimide (but curiously not iodoacetate or iodoacetamide) blocked the internalization of NBD-phosphatidylserine[29]. The compounds glycerophosphoserine and glycerophosphoethanolamine (which result from the deacylation of the respective lipids) also inhibited internalization. Most striking is the observation that the internalization of NBD-phosphatidylserine is stereospecific and fails to occur with D-NBD-phosphatidylserine. Other investigators using erythrocytes and examining the internalization of either spin-labeled or diacyl species of phosphatidylserine have identified the transbilayer movement of the lipid as an ATP-dependent process that is sensitive to sulfhydryl modifying reagents[7,11,43,69,70]. (More detailed discussion of these data is given in the article by Devaux and Zachowski in this issue)[12].

Additional lines of experimentation using mutant CHO-K1 cells defective in phosphatidylserine synthase provided evidence that phosphatidylserine is taken up by these cells and transported to the inner mitochondrial membrane where it is decarboxylated to form phosphatidylethanolamine[65]. Although these latter studies have not provided any details of the mechanisms involved (i.e., endocytosis or transbilayer movement of the lipid followed by transport), the results provide unambiguous evidence for routes of phosphatidylserine transport between the plasma membrane and the inner mitochondrial membrane. This translocation process occurs in wild type as well as mutant cells and can play a role in the regulation of the activity of phosphatidylserine synthase[31].

Phosphatidic acid

The NBD-analog of phosphatidic acid readily inserts into the plasma membrane of Chinese hamster fibroblasts at 2 °C. Unlike other fluorescent lipid analogs the NBD-phosphatidic acid is rapidly metabolized to NBD-diacylglycerol and disseminated throughout the cell at 2 °C[35]. Shifting the cells to 37 °C leads to extremely rapid degradation of the NBD-diacylglycerol (approximately 50 % of the fluorescent glycerolipid being converted to NBD fatty acid, that is lost from the cell within 10 min). The remaining NBD-diacylglycerol continues to be degraded and minor amounts are converted to NBD-phosphatidylcholine and NBD-triacylglycerol. The NBD-triacylglycerol subsequently accumulates in lipid droplets within the cell. Structural manipulation of NBD-phosphatidic acid and investigation of the subsequent metabolism has been revealing[34]. The phosphonate analog of NBD-phosphatidic acid, which contains a methylene group in place of the usual bridge oxygen between the glycerol backbone and the phosphorous atom, cannot be cleaved to yield NBD-diacylglycerol. When cells are incubated with the phosphonate analog only plasma membrane labeling is observed suggesting that the formation of diacylglycerol is required for the intracellular labeling to occur. Analogs that contain *sn*-1 palmitate, *sn*-2 phosphate and *sn*-3 NBD-fatty acid on the glycerol backbone are readily cleaved to yield the 1, 3 NBD-diacyl-*sn*-glycerol which is readily distributed throughout the cell in a manner similar to the 1 acyl 2 NBD-*sn*-glycerol. Furthermore, cleavage of NBD-phosphatidylcholine located in the outer leaflet of the plasma membrane, with phospholipase C generates NBD-diacylglycerol in situ; and this leads to the rapid fluorescent

labeling of intracellular organelles. These results demonstrate that NBD-diacylglycerol can readily undergo transbilayer movement at 2 °C and redistribution throughout the cell. The mechanism by which this redistribution through the cell occurs is not known but it is not due to the solubility and random partitioning of NBD-diacylglycerol because this lipid (unlike its more polar counterparts) has relatively low water solubility and does not rapidly exchange among membranes. Possible explanations of the rapid distribution of NBD-diacylglycerol include a soluble carrier for this lipid or zones of continuity or adhesion among different subcellular membranes that permit rapid lateral diffusion.

Sphingolipids

The movement of NBD-sphingomyelin from the outer leaflet of the plasma membrane to intracellular membranes has been investigated by Koval and Pagano[22]. Insertion of NBD-sphingomyelin into the outer leaflet of the plasma membrane of CHO-K1 occurs readily at 7 °C. Subsequent warming of these cells leads to internalization of the fluorescent lipid which yields prominent labeling of a central perinuclear zone. The internalization process is prevented by depleting cellular ATP levels with deoxyglucose and sodium azide. The initial labeling of the internal compartments does not occur in either the Golgi apparatus or the lysosomes but in a region identified by independent criteria as that closely associated with the centrioles. With increasing incubation times the Golgi apparatus also becomes labeled as a consequence of the metabolism of the NBD-sphingomyelin to NBD-ceramide (discussed above in the section on transport of sphingolipids to the plasma membrane). Since the centrioles are a microtubule organizing center the effects of nocadazole upon the centriolar accretion of internalized NBD-sphingomyelin were investigated. The disruption of microtubules with nocodazole did not prevent the internalization of NBD-sphingomyelin but dramatically changed the distribution of the fluorescence from that of a centrally concentrated zone to a random peripheral distribution. These latter results indicate that microtubules play a role in directing vesicle cycling to and from the plasma membrane. The t½ for round trip cycling of NBD-sphingomyelin from the plasma membrane to the intracellular compartment and back is approximately 40 min. The different results obtained with NBD-sphingomyelin given exogenously and NBD-sphingomyelin generated in situ (synthesized from exogenous NBD-ceramide) are intriguing. The results indicate that the assembly of nascent NBD-sphingomyelin into the plasma membrane is spatially segregated from the recycling of NBD-sphingomyelin to and from the plasma membrane.

Gangliosides can also be effectively 'transplanted' into the outer leaflet of the plasma membrane of tissue culture cells[52]. When polarized epithelial cells are labeled with fluorescent gangliosides at the apical membrane there is no detectable movement of the lipid across the tight junction to the basolateral surface. Additional evidence suggests that endogenously synthesized gangliosides may be selectively transported to the apical membrane to the exclusion of the basolateral membrane.

Cholesterol

Several investigators have introduced cholesterol into the outer leaflet of the plasma membrane by exchange from lipid vesicles and examined subsequent internalization and esterification. In two studies the exogenously introduced cholesterol failed to equilibrate with the endogenously synthesized cholesterol as judged by the subsequent esterification of the endogenous pool but not the exogenous pool[24,37]. However, in smooth muscle cells and lung fibroblasts, low levels of cholesterol taken up by the cells (i.e. < 5 %) can undergo esterification at very slow rates[50,51]. Additional evidence has been presented to suggest that very slow mixing (i.e., requiring 24 h) of exogenous vesicle-derived cholesterol can occur with LDL-derived pools[40]. Direct evidence has been presented demonstrating that plasma membrane cholesterol readily enters the endosomal pool[15] but this cholesterol appears to be largely sequestered from entering other cellular pools and may be recycled to the plasma membrane via the trans Golgi[8].

Lipid transport to the mitochondria

Phosphatidylcholine

Newly synthesized phosphatidylcholine is rapidly transferred from its site of synthesis to the outer mitochondrial membrane[68]. The estimated radioequilibration time using a ^{14}C-choline precursor is approximately 5 min. In contrast the movement of labeled phosphatidylcholine from the outer to the inner mitochondrial membrane is a much slower process. A brief (15 min) labeling of hepatocytes with ^{3}H-choline followed by separation and analysis of the outer and inner mitochondrial membranes demonstrates that the specific activity of phosphatidylcholine in the outer membrane is 9.5 times that of the inner membrane. Thus the pools of newly synthesized phosphatidylcholine at the endoplasmic reticulum appear to be in rapid equilibrium with both the plasma membrane (see section on lipid transport to the cell surface) and the outer mitochondrial membrane, but not the inner mitochondrial membrane.

Phosphatidylethanolamine

In contrast to phosphatidylcholine, newly synthesized phosphatidylethanolamine is transferred to the mitochondria very slowly[68]. The radioequilibration of microsomal and mitochondrial membranes requires at least 2 h of chase in the presence of excess unlabeled ethanolamine following a 15-min pulse labeling. The mechanism by which phosphatidylethanolamine is transported to the

mitochondria is unknown and there has been no characterization of the sensitivity to metabolic inhibitors.

Phosphatidylserine

Phosphatidylserine transport to the mitochondria has been studied by exploiting the fact that the enzyme phosphatidylserine decarboxylase is located at the inner mitochondrial membrane[10,56]. When phosphaditylserine arrives at the inner membrane of the mitochondria it is rapidly decarboxylated to yield phosphatidylethanolamine. The synthesis of phosphatidylserine occurs primarily in the endoplasmic reticulum but significant activity has also been found in both Golgi and nuclear membrane preparations[16,54,56]. Thus by judicious use of pulse-chase protocols with a radiolabeled serine precursor it is possible to follow the translocation and import of phosphatidylserine into the mitochondria by the appearance of radioactivity in phosphatidylethanolamine. In some cell lines it is now apparent that the translocation of phosphatidylserine to the mitochondria followed by its decarboxylation is a major pathway for the synthesis of phosphatidylethanolamine[30,31,61,65]. This result demonstrates that significant amounts of phosphatidylethanolamine found in cell membranes must be derived from the mitochondria.

The translocation of phosphatidylserine from its site of synthesis to the mitochondria can be inhibited by depletion of cellular ATP with sodium azide plus sodium fluoride[62]. Subcellular fractionation reveals that phosphatidylserine accumulates in the microsomal fraction of poisoned cells suggesting that ATP is required for an early step in the transport process[62]. The import of phosphatidylserine into the mitochondria was further investigated using isolated subcellular fractions from rat liver. The synthesis, translocation and decarboxylation of phosphatidylserine occurs readily in cell-free homogenates derived from rat liver[63]. The phosphatidylserine synthase reaction requires microsomal membranes whereas the decarboxylase reaction requires mitochondria. Incubation of microsomes containing radiolabeled phosphatidylserine, with mitochondria, led to the decarboxylation of the lipid in vitro. The translocation of phosphatidylserine from the microsomes and its import into the mitochondria does not require ATP. The transport reactions are saturable with respect to the amount of microsomes added to the reaction and the data are consistent with the collision-based transfer of phosphatidylserine from microsomes to mitochondria. Kinetic analysis of the transport indicates that the rate-limiting step in the in vitro process is the movement of phosphatidylserine from the microsomes to the mitochondria and not the intramitochondrial movement of the lipid or the decarboxylation reaction.

The disparity between the results obtained with phosphatidylserine transport in intact cells (ATP requiring)[62] and the results obtained in vitro with isolated organelles (ATP independent)[63] is subject to several interpretations. One hypothesis favored by Voelker is that phosphatidylserine translocation to the mitochondria is a two-step process: the first step requiring ATP and the second (collision-based transfer) step being ATP independent. In this interpretation only the second step would be reconstituted with isolated organelles. Recent evidence obtained with permeabilized cells suggests that these interpretations are correct[64]. When CHO-K1 cells are permeabilized with saponin after a pulse labeling with [^{3}H]-serine there is essentially no decarboxylation of newly synthesized phosphatidylserine. However, if the pulse-labeled cells are supplemented with ATP after permeabilization, they decarboxylate the nascent phosphatidylserine at rates which are comparable to those in intact cells for a period of 2 h. The translocation-dependent decarboxylation of phosphatidylserine in permeabilized cells is optimal with 2 mM ATP supplementation but also occurs with high levels of GTP, CTP, UTP, and ADP. Significantly, AMP and the non-hydrolyzable analog of ATP, AMPPNP, fail to support the translocation of phosphatidylserine.

The mechanism by which phosphatidylserine is transported to the mitochondria is unclear. The ATP requirement for the process would be consistent with a vesicle transport process or some interaction with cytoskeletal elements or both. However, commonly used inhibitors that interfere with these processes (such as monensin, nocadazole and cytocholasin D) fail to alter the translocation-dependent decarboxylation of phosphatidylserine in the intact cell[62,64]. The data obtained with isolated organelles indicate that the final steps of phosphatidylserine import into the mitochondria can occur in an ATP-dependent manner. Collectively these data suggest that phosphatidylserine translocation to the mitochondria is a two-step process with the first step requiring ATP to place phosphatidylserine in an environment that is permissive for the second step, the collision-based transfer of the lipid to the mitochondria.

Cholesterol

The general exclusion of cholesterol from mitochondria occurs in many cell types. However, in adrenal tissue there is clear evidence that cholesterol is imported into the mitochondria for the initial steps in steroid hormone biosynthesis. The transport of cholesterol from the endoplasmic reticulum or intracellular storage droplets to the mitochondria can be inhibited by cytocholasin B and vinblastine[6]. The transport of cholesterol to the mitochondria appears to continue even in the absence of metabolism and is stimulated by ACTH[23]. Additional evidence has been presented to indicate the presence of an intramitochondrial transporter for cholesterol that is inhibited by cholesterol sulfate[23]. Several cell types also express a sterol carrier protein that has been shown in in vitro experiments to facilitate the movement of monomeric cholesterol from lipid droplets isolated from adrenal tissue to mitochondria from the same source[4].

Lipid transport from the mitochondria

Phosphatidylethanolamine

With the demonstration that the decarboxylation of phosphatidylserine in the mitochondria could account for a large portion of the synthesis of phosphatidylethanolamine in some cells[30,31,61,65] it has become apparent that a significant amount of phosphaditylethanolamine must be exported from the mitochondria to other organelles. One early study[62] provided some data demonstrating that phosphatidylethanolamine synthesized in the mitochondria could be recovered in microsomes derived from BHK fibroblasts. However, one of the most striking lines of experimental evidence appears in studies by Vance and Vance which show that phosphatidylethanolamine made by the decarboxylation of phosphatidylserine in the mitochondria is used in the biogenesis of lipoprotein particles (VLDL) destined for secretion[55]. At present there is no information available to indicate the mechanisms whereby phosphatidylethanolamine is disseminated from the mitochondria to other subcellular organelles.

Lipid transport at the endoplasmic reticulum

From numerous studies localizing the lipid biosynthetic apparatus at the endoplasmic reticulum[3,16,54,56], it is clear that significant export of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and cholesterol must occur. Of the aforementioned lipids the least is known about phosphatidylinositol transport. At present it is not clear how much of this transport is directed through other organelles such as the Golgi and how much occurs via vesicles that may bud from the endoplasmic reticulum and move directly to target organelles. The role of lipid exchange/transfer proteins[66,67] in these processes has also remained ambiguous.

Within the endoplasmic reticulum it is evident that transbilayer translocation of lipid can occur. Precise details of this transbilayer movement are still lacking but Bishop and Bell[3] have provided evidence that a specific phosphatidylcholine transporter exists in microsomal membranes. The activity of the transporter is ATP-independent, stereospecific and sensitive to proteases and protein modifying agents. The transporter is also capable of using lysophosphatidylcholine as a substrate[19]. Backer and Davidowicz[1] have reported the solubilization and reconstitution of the microsomal phosphatidylcholine transporter from rat liver, and their investigations have laid the groundwork for the purification of this protein.

Future directions

Much has been learned about general aspects of lipid translocation in animal cells over the last 5 years. There is clear evidence for both ATP-dependent and ATP-independent processes as well as vesicle-based transport. Specific details are still lacking in virtually all areas of lipid translocation. The exact role played by lipid exchange/transfer proteins needs to be addressed by genetic experiments. Likewise the nature of lipid transport vesicles needs to be elucidated to establish if these vesicles are used for protein translocation or represent unique populations. In addition, the transport routes of different lipid classes such as cholesterol and sphingomyelin needs to be studied in detail to determine commonality as well as divergence in these pathways. Elucidation of the biochemical details of lipid transport will require more sophisticated reconstitution experiments with isolated organelles and permeabilized cells. One of the greatest needs of this field is for good genetic methods to isolate mutants defective in lipid transport. The availability of such mutants will enable genetic complementation and molecular cloning techniques to define the number of genes involved in these processes and will ultimately yield structures of the molecules involved.

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Lipid transfer in plants

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Summary. Plant cells contain cytosolic proteins, called lipid transfer proteins (LTP), which are able to facilitate in vitro intermembrane transfer of phospholipids. Proteins of this kind from three plants, purified to homogeneity, have several properties in common: molecular mass around 9 kDa, high isoelectric point, lack of specificity for phospholipids, and binding ability for fatty acids. The comparison of their amino acid sequences revealed striking homologies and conserved domains which are probably involved in their function as LTPs. These proteins could play a major role in membrane biogenesis by conveying phospholipids from their site of biosynthesis to membranes unable to form these lipids. Immunochemical methods were used to establish an in vivo correlation between membrane biogenesis and the level of LTP or the amount of LTP synthesized in vitro from mRNAs. The recent isolation of a full-length cDNA allows novel approaches to studying the participation of LTPs in the biogenesis of plant cell membranes.
Key words. Lipids; phospholipids; galactolipids; fatty acids; transfer; binding; membranes; mitochondria; chloroplasts; biogenesis.

Introduction

Lipid dynamics play a major role in membrane biogenesis. The need for intracellular lipid movements, demonstrated for several living cells, is based on the observation that the enzymes involved in the biosynthesis of some lipids are localized in specific membranes and compartments, although the lipids synthesized are distributed among all the membranes of the cells. This is also true for plant cells which contain, in addition to organelles similar to those of other eucaryotic cells, some characteristic ones, like plastids. Studies on plant lipid metabolism [18,19] have concluded that such intracellular translocation of lipids must occur. For example, phosphatidylcholine (PC), which is the major phospholipid of the envelope membranes of chloroplasts as well as of mitochondrial membranes, is not synthesized by these membranes; in contrast, the endoplasmic reticulum contains all the enzymes needed for the de novo synthesis of this lipid [18,19]. The same observation has been made for other phospholipids, like phosphatidylethanolamine (PE) or phosphatidylinositol (PI). Moreover, movements of lipids within organelles are needed since, for example, galactolipids which accumulate in thylacoid membranes of chloroplasts are synthesized in the envelope; an intrachloroplastidial transfer of these lipids from the envelope to the thylacoids therefore seems to be necessary [12] (fig. 1).

In addition to this need for membrane biogenesis, intracellular movements of lipids are strongly suggested by the cooperative pathway assumed to occur between the endoplasmic reticulum and the plastid for the biosynthesis of galactolipids containing polyunsaturated fatty acids. This pathway involves an exportation of acyl chains (mainly in the form of oleyl-CoA), synthesized within the plastid, towards the endoplasmic reticulum. The desaturation reaction which takes place in this membrane leads to the formation of diunsaturated acyl chains which are transferred to the plastid as phosphatidylcholine molecules; the final desaturation step, which occurs within the plastid, allows the synthesis of galactolipids containing triunsaturated fatty acids. In this rather complicated pathway, the movements of lipids between the endoplasmic reticulum and the plastid play a major role (fig. 1).

What are the mechanisms of these intracellular fluxes of lipids? A first possibility is a spontaneous movement of lipids as free molecules. However, the weak solubility of phospholipids in the aqueous phase leads to the movement of these molecules being very slow [21,50]. A second mechanism is the transfer of membrane vesicles from the endoplasmic reticulum to other membranes through the Golgi membranes, according to the membrane flux theory [28]. An argument in favor of this hypothesis in plant