Lipid transfer proteins are critical elements in the regulation of the body's access to lipid nutrients, to their disposition via plasma lipoprotein composition and uptake/egress into/from various cells and tissues. They are also central to the organization and composition of cellular organelles, which are bounded by lipid membranes. Included in this series of reviews is a set that covers a variety of physiological processes.

OVERVIEW OF TRANSFER PROTEINS REVIEWED IN THIS SERIES

Lipids, for the purpose of these reviews, include free fatty acids, neutral glycerides, free cholesterol, cholesteryl esters, phosphoglycerides, sphingolipids, and glycolipids. None of these lipids have significant aqueous solubility and so their carriage into and through the tissues requires their association with proteins, many of which would be categorized as lipid transfer proteins. Some of the proteins with which the complex lipids associate are plasma lipoproteins, such as chylomicrons, chylomicron remnants, VLDL, and LDL. Although these lipoproteins are remodeled in the plasma by transfer proteins, their uptake into cells and tissues is mediated by specific receptors; for example, LDL receptor. These receptors are not normally categorized as transfer proteins, although they do in fact very effectively transfer lipids into cells. The distinction between receptors and transfer proteins is not in all cases unambiguous. For example, the scavenger receptor, CD36 (SRB-2), may function either as a conventional receptor or as a transfer protein. The lipid transfer proteins primarily recognize lipid, while the receptors recognize protein as the primary ligand.

In the series, there are nine reviews, which may be categorized into three groups. In the first group are reviews discussing those transfer proteins that mediate the assembly and metabolism of lipoproteins and that mediate the transfer of lipids from the diet to the tissue and from tissue to tissue. The second group of reviews discusses transfer proteins engaged in the distribution of lipids within the cell and that may modify the lipid composition of individual subcellular membranes. The final review discusses a special class of lipid transfer proteins, those of plants which facilitate the creation of water-proof barriers of plant cells.

TRANSFER OF LIPID FROM DIET TO TISSUE

Dietary lipid esters, mostly in the form of triglycerides and cholesteryl esters, are hydrolyzed in the lumen, yielding free fatty acids and free cholesterol. These products enter the enterocyte, either by diffusion or facilitated transport, where they are resynthesized into triglycerides and cholesteryl esters. These processes represent mechanisms for the transport of complex lipids across the relatively impermeable apical membrane. The triglycerides are either packaged into chylomicrons, a lipid rich lipoprotein containing the unique nonexchangeable apolipoprotein, apo B48, or are stored as lipid droplets, which may be later mobilized for the assembly of chylomicrons. The noncovalent coupling of glycerides with apo B48 occurs cotranslationally to initiate the assembly of chylomicrons and is mediated by the lipid transfer protein, microsomal triglyceride transfer protein (MTTP), the function of which has been reviewed by Hussain and Sirwi (1). A deficiency of MTTP results in abetalipoproteinemia, the disease associated with low plasma lipids and vitamin A deficiency. MTTP has other functions, exemplified by its role in the loading of the lipid antigen presenting molecule [CD1, reviewed by Teyton (2); see below]. The lipids loaded here are much more complex sphingolipids, not triglycerides, illustrating the quite broad ligand specificity of this transfer protein. The surface of the chylomicron is covered by a monolayer of phospholipid, which is added through the mediation of the phospholipid transfer protein.
protein (PLTP), one of the functions of which is to provide much of this covering. Our knowledge of the PLTP is reviewed by Jiang (3). The enterocyte is one of the sources of this protein. The handling of cholesterol and other sterols by the intestine is also quite complex. The free cholesterol present in the lumen and released by the hydrolysis of dietary cholesteryl ester is taken up into the enterocyte, mediated by a transfer protein, Niemann-Pick C1 Like-1 (NPC1L1), which is the target of the cholesterol-lowering drug, ezetimibe. Some of the cholesterol is reesterified and packaged in the core of chylomicrons. The enterocyte is also a source of HDL, here generated via the action of the ABCA1 transporter providing phospholipid and cholesterol to the nascent HDL. About 30% of the plasma HDL derives from the intestine. The transfer function of ABCA1 is reviewed by Phillips (4). It participates in the formation of nascent HDL by intestine, hepatocyte, and cholesteryl-loaded peripheral cells; for example, macrophage foam cells. For the latter transfer, it is a major component of “reverse cholesterol transport”. Deficiency of this transporter results in Tangier’s disease, in which low plasma HDL and LDL is associated with cholesteryl ester-loaded lymphoid tissue. ABCA1 is one member of a large family of ABC transporters, 48 in number, of which 20 family members are engaged in lipid transport in various physiological roles (5). Some are intracellular lipid transport proteins. Dietary sterols include cholesterol and many different sterols derived from plants and other invertebrates (xenosterols). In the enterocyte are the two sterol transporters, ABCG5/G8, that pump sterols into the lumen, protecting the organism from the potential toxicity of the xenosterols. The function, regulation, and pathophysiology of these transporters is reviewed by Patel and colleagues (6). This transporter complex is also involved in the secretion of cholesterol from the hepatocyte into the bile and of excess cholesterol from the enterocyte. The absence of this transporter results in the absorption of plant sterols—the entity known as sitosterolemia.

The plasma lipoproteins are complexes made up of a core of hydrophobic lipids, triglyceride, and/or cholesterol ester, with a surface containing amphipathic phospholipids, free cholesterol, nonexchangeable apo B, and a variety of exchangeable apoproteins. These components may be acquired intracellularly during the biogenesis of the lipoproteins. Indeed, for example, in the absence of PLTP in the liver, secretion of lipoproteins may be attenuated. PLTP may catalyze the exchange of phospholipids among circulating plasma lipoproteins. The phospholipid transfer protein, the properties of which are reviewed by Jiang (3), is synthesized in a variety of tissues, notably liver, adipose tissue, lung, and macrophages. Each of the first three tissues may contribute about 20% of the circulating activity.

Circulating plasma lipoproteins are remodeled not only by the exchange of their surface components, but also by their core components, mediated by the cholesteryl ester transfer protein (CETP), the properties of which are summarized by Rye and her colleagues (7). Thus, the triglycerides of the triglyceride-rich lipoproteins are exchanged for cholesteryl esters of the smaller lipoproteins, notably HDL. This transfer protein has excited a good deal of interest among members of the cardiovascular research community. This is because of two aspects of the study of HDL metabolism. First, it was widely held that HDL is an atheroprotective lipoprotein, so that strategies for the clinical elevation of HDL have been much sought. Second, individual patients and families lacking CETP had notably elevated HDL. It was natural then that the use of inhibitors of CETP may be deployed to treat those at high risk of developing atherothrombotic cardiovascular disease. This has been put to the test in several clinical trials with mixed results, mostly because of the appearance of untoward side effects.

The function of the plasma lipoproteins is primarily to distribute lipids to various tissues for energy, metabolism, specialized product formation, and structure of cells and tissues. The transfer proteins just discussed are mainly involved in the fashioning of lipoprotein structure for these functions. The intact apo B lipoproteins are internalized by endocytic receptors, like the LDL receptor. On the other hand, the triglyceride-rich lipoproteins are metabolized while in the plasma and extracellular fluid by lipoprotein lipase, mostly of muscle, skeletal and cardiac, and adipose tissue, liberating free fatty acids, which are used either as an energy substrate or for storage. The transport of these fatty acids into the requiring tissues is facilitated by the fatty acid transporter, CD36, present in the plasma membrane. This role of CD36 is reviewed by Glatz and colleagues (8). CD36 is a member of the family of class B scavenger receptors (SRB-2). CD36 on its own is not sufficient to mediate fatty acid transport but collaborates with cytoplasmic fatty acid binding protein/s. The interaction of CD36 with fatty acid has been clarified by the recent publication of its crystal structure (9). It contains hydrophobic pockets to which fatty acids may be bound. This study also described the interaction of CD36 with a protein of Plasmodium falciparum, an interaction that interrupts its binding of OxlDL, another of its potential ligands. OxlDL may enter macrophage foam cells through this receptor, contributing to atherogenesis. Like other members of the lipid transport protein family, this protein has multiple functions. It has broad specificity for ligand. It is widely expressed in muscle, adipose tissue, enterocytes, endothelial cells, monocyte/macrophages, and platelets, in each of which it may manifest distinct functions.

A second member of the scavenger receptor class B family is SR-B1, which is involved in the movement of cholesterol and its esters across membranes, often without the accompanying holo-lipoprotein, the process known as selective cholesteryl ester uptake. The knowledge on this receptor is reviewed by Azhar and colleagues (10). It was the first “HDL” receptor identified, as its uptake of cholesteryl ester is mainly from HDL. In the liver, the transferred cholesterol is converted to bile acids, while in the steroidogenic cells, steroid hormones are the products. Like other transfer proteins, it possesses a hydrophobic tunnel into which fits its ligands. Also like other transfer proteins, it does not have a high specificity for ligand. In addition to
selective transfer, it mediates the bidirectional movement of cholesterol and participates in reverse cholesterol transport, as for example, in macrophages, where it is also expressed. HDL interacting with SR-B1 has the capacity to activate endothelial nitric oxide synthase. The knockout of this receptor in the background of apoE deficiency results in the most dramatic model of occlusive coronary atherosclerosis and myocardial infarction in mice (11). This receptor is expressed in many nonmammalian vertebrates. A recent publication notes the effect of SR-B1 mutation in white canaries, attributable to the inability to transfer dietary carotenoids into the birds’ wings (12).

TRANSFER OF PHOSPHOLIPIDS AND SPHINGOLIPIDS AMONG CELLULAR MEMBRANES

The cell membranes are composed of plasma membrane and the membranes of several intracellular organelles. These are made up predominantly of bilayers of phospholipids, cholesterol, and glycolipids. The intracellular membranes include those of endoplasmic reticulum, endosomes, mitochondria, and Golgi apparatus. Each of the cellular compartments is delimited by a bilayer membrane of distinctive composition. The major phospholipids are phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol (PI) sphingomyelin (SM), and cardiolipin (CL). They are not uniformly distributed among the intracellular membranes (13). PC is the major phospholipid of each of these membranes, followed by PE and SM. However, there are distinctive features. Thus the plasma membrane is enriched in PS and SM and in free cholesterol. The Golgi is relatively high in SM. Essentially all of the cell CL is in the inner mitochondrial membrane, the site of its biosynthesis. How do these compositional differences come about? Nonvesicular transport (14) from the sites of lipid biosynthesis involving lipid transfer proteins is a major mechanism. The lipid transfer proteins may carry monomeric lipids, either via the soluble matrix or by binding to particular membranes, on- or off-loading their lipid ligands, which is probably facilitated at membrane contact sites (15). While there are several lipid transfer proteins, there are relatively few well-researched examples. Among these is the transfer of ceramide (CERT), which is reviewed by Hanada (16). The ceramides are precursors of SMs and the glycosphingolipids. Glycosphingolipids and glycolipids are the major forms of exogenous (e.g., mycobacterial) or endogenous lipid antigens, the presentation of which to activate NKT cells is reviewed by Teyton (2). These lipid antigens are ultimately presented to the NKT cells loaded on the CD1 molecule (MHC class I type). But the trajectory between the source, either exogenous or endogenous, mostly through the endosomal compartment and the cell surface loaded CD1 molecule, involves a succession of lipid transfer proteins, MTTP, NPC2, and four small lipid transfer proteins. The latter four proteins, the saposins, are derived from a common precursor, prosaposin. The saposins are thought to facilitate the hydrolysis of the lipid antigen in the lysosome, although their precise function is poorly understood. Defects in these transfer proteins may impair the ability to mount an immune response to exogenous lipid antigens.

A couple of transfer processes, not included in this review series, contribute to the differential composition of organelar membranes. First is the enrichment of the plasma membrane with PS. A major portion of this transfer occurs at the membrane contact sites between endoplasmic membrane and plasma membrane, where the oxysterol binding protein related protein (ORP5/8) mediates the counter transport of PI4 phosphate and PS (17). This set of proteins is also involved in the transport of PS at a contact site between the endoplasmic reticulum and the outer mitochondrial membrane (18). The transferred PS is decarboxylated at the outer face of the inner mitochondrial membrane, to which it is transported through the intermembrane space by the lipid transfer complex Ups2-Mdm35 (19). The exchange of phospholipid between the endoplasmic reticulum and the mitochondria was described 50 years ago (20). The decarboxylated PS yields PE, which is the major pathway for mitochondrial PE formation and is essential for mitochondrial function. The other functionally important mitochondrial phospholipid, which is formed in the mitochondria, is CL. The pathway from phosphatidic acid (PA) to CL involves the cooperative action of the endoplasmic reticulum and the mitochondria, and has been recently reviewed in the Journal of Lipid Research (15). This requires the transport of PA from the outer to the inner mitochondrial membrane through the intermembrane space. The lipid transfer protein complex in this case is Ups1-Mdm35 (21). CL is unique among phospholipids in that 80% of its four fatty acyl groups is made up of linoleic acid (18-2), and, interestingly, with very little arachidonic acid (22). This fatty acid composition comes after its biosynthesis by remodeling via a transacylation enhanced by the mitochondrial protein, tafazzin. The precise mechanisms require further study (23).

Cell membrane asymmetry is best exemplified in the plasma membrane, the cytoplasmic face of which is enriched in PS and PE, while the outer face is rich in SM and glycolipids. The asymmetry is maintained by the ATP-dependent flippase, P-4 ATPase (24). This is important, as PS on the outer leaflet is a signal for the uptake of apoptotic cells (efferocytosis). PS moves to the outer leaflet in apoptotic cells when caspase inactivates the flippase, while activating the scramblase (25).

TRANSFER OF CHOLESTEROL AMONG CELLULAR MEMBRANES

Cholesterol is a critical component of cellular membranes. The distribution of cholesterol among different membranes is not uniform. By far, the highest ratio of cholesterol to phospholipids is in the plasma membrane. The trajectory of cholesterol among the cellular membranes is quite complex with many elements yet to be characterized.
Membrane cholesterol may derive from endogenous biosynthesis in the endoplasmic reticulum, or from circulating lipoproteins through the cell surface receptors, and following endosomal pathways. Cholesterol from these sources may be transported to other membranes, most notably, the plasma membrane and mitochondria. This transport may be vesicular or nonvesicular, mediated by lipid transfer proteins operating through soluble compartments or at membrane contact sites. Cholesterol homeostasis is tightly regulated and its movement between membranes is influenced by the composition of the donor and acceptor membranes (26). Following the internalization of LDL through its surface receptor, hydrolysis of its contained cholesteryl ester yields free cholesterol, which may be exported using the transfer proteins NPC1 and NPC2 (Niemann Pick type C proteins) to the endoplasmic reticulum. In the absence of these proteins, lipids accumulate in the late endosome/lysosome in the lipid storage disease, Niemann Pick disease.

There are two other transfer protein sets involved in cholesterol movement. One set, the ORP discussed above in relation to PS enrichment, may transfer cholesterol to the plasma membrane. There are 12 family members in mammalian cells (27). The other set are the StARD proteins, containing START (steroidogenic acute regulatory protein-related lipid transfer domain protein), of which there are 15 family members. Some of these proteins are involved in steroidogenesis. In steroidogenic cells, endosomal cholesterol may be transferred to mitochondria, mediated by NPC2, MNLA4 (a StAR domain protein), and StARD3. Reverse transport from the plasma membrane into the cell involves StARD4.

Lipid transfer protein activity is conserved through evolution, as represented by their activity in plants, as reviewed by Edqvist and colleagues (28). In summary, this series describes the behavior of some of the lipid transfer proteins. They have the capacity to transfer a variety of relatively insoluble lipids, carried as monomers, either through soluble matrices or at more closely applied membrane contact sites. They transfer lipids among lipoproteins or from cells to lipoproteins, or between their sites of synthesis and selected membranes. Their three-dimensional structure describes channels that accommodate the transported lipids. They often alter conformation as they acquire the lipid ligands, and again as they release the lipids. They exhibit relatively modest specificity for hydrophobic lipid ligands.

Some of the transfer proteins covered in this series appear to have multiple functions, some of which may not be transport functions. Clearly, these proteins play important roles inorganistic and cellular homeostasis. There is much study that remains to uncover the roles of these as-yet-to-be characterized proteins.

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