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Concluding remarks and perspectives

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The existence of subcellular compartments provides a spatial separation of metabolic events and cellular processes, which allows the coexistence and coordination of different pathways within the cell. Biological membranes form the barrier between organelles and their environment. As well as proteins, lipids are integral parts of all cellular membranes. They act as structural elements, but by providing the proper environment they also affect the activity of enzymes present in membranes by modulation. The elucidation of processes involved in the assembly of lipids into cellular membranes is therefore an important subject of modern cell biology.

The present Multi-author Review summarizes our knowledge of the supply of lipids to membranes, of mechanisms involved in intracellular lipid translocation, and of regulatory effects on the migration of lipids within the cell. Morton and Vance and Vance describe the interaction between extracellular (lipoproteins) and cellular lipids. A plasma lipid transfer protein catalyzes the migration of cholesterol esters and triacylglycerols between lipoproteins and cells, which leads to the modulation of cellular lipid levels and directly influences cellular membrane lipid composition. Vice versa, cellular lipid metabolism affects the assembly and the secretion of lipoproteins into the plasma. Vance and Vance argue that preferential assembly of newly synthesized lipids into lipoproteins acts as the driving force for the secretion of lipoproteins from the endoplasmic reticulum via the Golgi apparatus to the cell surface.

Lipids either taken up or synthesized by cells must be distributed correctly among cellular membranes. The unique lipid composition of each cellular membrane necessitates efficient and well-balanced processes for the supply of membrane lipids to organelles. Routes and mechanisms of lipid transport have been studied in mammalian cells (Voelker), plant cells (Arondel and Kader) and microorganisms (Daum and Paltauf). Biochemical, cytological and molecular biological techniques were employed to study the process of lipid translocation and the components involved. Correlation between results obtained in vitro and in vivo is one of the crucial problems in all systems.

Intracellular transport of distinct lipid classes is the subject of articles presented by Wirtz and Gadella, Reinhart and Sasaki. The existence of soluble lipid transfer proteins, which are more or less specific for phospholipids, sterols and/or glycolipids, offers an attractive mechanism for lipid migration between organelles. Analogously, fatty acid binding proteins (Kaikaus et al.) seem to be involved in the uptake, transport and metabolism of fatty acids. Fatty acid binding proteins are abundantly present in mammalian cells, but not in prokaryotes and lower eukaryotes, e.g. yeast. Lipid transfer proteins and fatty acid binding proteins have been studied very intensively with respect to their molecular structure and their lipid binding properties.

Another possible mechanism of intracellular lipid migration is vesicle flow. This process is known to be utilized for the delivery of secretory proteins from internal membranes to the plasma membrane. Much of the existing data on intracellular transport of sterols and also of glycosphingolipids is consistent with a vesicular mechanism. The involvement of vesicle flow in phospholipid transport is still a matter of dispute.

Besides protein-catalyzed lipid transfer and vesicle flux, membrane fusion is a conceivable mechanism for lipid translocation between subcellular membrane fractions. Burger and Verkleij point out the advantages of artificial model systems versus biological systems when studying this process, but also draw attention to the dangers involved. Membrane fusion seems to be a very fast local point event, which is under strict control. Both non-bilayer-forming lipids and proteins might be involved in destabilizing the bilayer of a membrane. The existence and the mode of action of putative fusion factors remain to be demonstrated.

Transmembrane movement of lipids (Zachowski and Devaux) is a very important step in intracellular lipid migration. How lipids transverse the membrane depends very much on their properties, especially their charge. Passive transport (diffusion), facilitated transport catalyzed by flippases, or active transport of lipids with the aid of translocases are possible mechanisms. The generation of membrane lipid asymmetry as a consequence of transbilayer movement (flip-flop) of lipids might be of importance not only for further transportation of lipids to other membrane systems, but also for cell-cell interaction or organelle contact.

Enormous progress has been made in the investigations of lipid traffic and assembly of lipids into membranes during the last years. Nevertheless, the authors of this Multi-author Review raise many important questions which remain unanswered so far. First a more precise knowledge of the localization of lipids (lipid composition of organelles, orientation of lipids within membranes) and of lipid synthesizing enzymes in cellular membranes will be necessary. This question sounds very simple, but only sophisticated techniques of cell fractionation and cytology will allow us to establish a more elaborate picture of the exact cellular destination(s) of lipids, and of the starting points for their movement between organelles. As an example, the so-called 'microsomes', which are generally accepted as the fraction with the major capacity for lipid synthesis, are certainly a mixture of various membrane populations. These subfractions will have to be defined by their (different?) biochemical or structural properties.

Then, of course, interest will focus on the clarification of mechanisms governing lipid migration. As already mentioned above, mechanisms working in vitro will have to be tested for their relevance in the living cell. What are the physiological roles of lipid transfer proteins, fatty acid binding proteins, flippases and translocases? Are these proteins involved in the biogenesis of organelle membranes, in the maintenance of membrane integrity, in membrane recycling, or – probably under the control of a mechanism as yet unknown – in all of these processes? How do lipid transfer proteins and fatty acid binding proteins interact with lipid molecules integrated in a membrane? Can lipid transferring proteins distinguish between putative target membranes, and if they do so, how does recognition occur? Do specific lipid transfer vesicles (in contrast to endocytotic or exocytotic vesicles of protein transport) exist within the cell? Which protein(s) are involved in the formation of these vesicles, when they bud off from the donor membrane, and during their fusion with the target membrane? How can the energy requirement of vesicle flux, which is well known for vesicular protein transport, be explained? Is the active synthesis of lipids, or an energy-dependent migration of vesicles along the cytoskeleton, the driving force of vesicular lipid translocation? If we assume that specific lipid transport vesicles exist, how is the sorting and targeting of lipids governed? How is membrane fusion controlled? What are the mechanisms distinguishing between lipid and protein components to be transported after membrane fusion had occurred? Do specific barriers exist which allow passage of one or the other type of molecule? Do fusogens, which are known to act very efficiently in vitro, have a comparable effect in the living cell? Which cellular processes might be influenced by or linked to lipid transport? Can lipid transport processes modulate the activity of membrane-associated enzymes by supply of lipids to membranes? Is lipid translocation connected to regulatory responses of the cell? Very recently an involvement of lipid transport in the phosphatidylinositol cycle has been suggested. Do further, as yet unknown, linkages between cellular processes and supply of lipids to cellular membranes exist?

What are the future perspectives suggested by the authors of this Multi-author Review for elucidating processes and mechanisms of lipid traffic and assembly of lipids into membranes? Biochemical techniques will be needed to isolate macromolecules (proteins) involved in lipid transport, and to develop and improve concepts for biologically relevant translocation assays. The aim of these studies will be the reconstitution of lipid transport systems to learn more about each single component involved. Techniques of molecular biology will allow cloning of genes encoding for proteins participating in lipid migration processes. Based on these studies the investigation of the regulation of lipid traffic will be possible. Isolation or construction of mutants with defects in the lipid transport machinery will be of enormous help to investigate linkages between lipid transport and other cellular processes, e.g. lipid synthesis, protein synthesis, protein secretion, energy production or assembly of the cytoskeleton network. Using these mutants for biochemical and cell biological experiments in vivo, it should be possible to unravel the physiological role of proteins either catalyzing lipid translocation, governing vesicle flux or controlling membrane fusion in vitro.

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