What Cholesterol Is For

Cell Membrane Cholesterol and Diseases

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Table of contents

List of Figures and Tables

Introduction

Recently, cholesterol has been receiving a lot of attention in both medical and scientific circles. Checking the 'lipid profile' in blood tests is one of the mandatory tests, and if the level of 'bad cholesterol' (associated with LDL), either a low-fat diet or statins—blockers of cholesterol synthesis in the body—are habitually prescribed, and such treatment is considered a universal and reliable protection against atherosclerosis and cardiovascular disease. And if we remember that cholesterol was first isolated from gallstones[1], its reputation is completely ruined. Naturally, the question arises: if cholesterol is so harmful and even deadly, why is it so persistently produced by the body's cells? One of the standard answers is that cholesterol is necessary for the synthesis of steroid hormones, vitamin D and bile acids. But if that is the case, wouldn't it be easier to take all of this in pill form and completely block cholesterol synthesis with the now widely available and popular statins, thereby eliminating the risks associated with such 'dangerous' cholesterol?

This book examines the significance of cholesterol from the perspective of cell biology. A living cell is a unique system in which the functioning of its constituent molecules is interdependent and coordinated, and any disorder in this coordination disrupts the normal functioning of the cell and can be fatal to it. Every living cell,

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[1] In 1769, Poulet de la Salle obtained a dense white substance ('fat wax') with the properties of fats from gallstones. In its pure form, cholesterol was isolated by chemist, member of the National Convention and Minister of Education Antoine Fourcroix in 1789. In 1815, Michel Chevreul, who also isolated this compound, named it cholesterine ('chole' meaning bile and 'stereos' meaning solid). In 1859, Marcellin Berthelot proved that cholesterol belongs to the class of alcohols, after which the French renamed cholesterol 'cholesterol' (from Wikipedia).

both prokaryotic and eukaryotic, is surrounded by cell ('biological') membranes that provide a diffusion barrier and regulate the inflow and outflow of molecules across the cell boundary. It can be said that without a membrane, there is no cell. Despite its apparent simplicity – for example, compared to the nucleus, which stores genetic information – the outer cell membrane (termed plasma membrane) ensures both the preservation of all cell contents and the continuous interaction of the cell with the external environment – from the delivery of nutrients to the reception and generation of various signals – chemical, electrical, mechanical, etc. Moreover, since in a multicellular animal organism all cells have the same genome, i.e., a set of genes derived from a single original cell (zygote), without the participation of cell membranes, which ensure the interaction of cells both with each other and with the external environment, it is hardly possible to explain the further differentiation of cells, i.e., their transformation into the most diverse cells of the organism – neurons, muscle and receptor cells, skin cells and all the rest. But this is another topic, still full of mysteries and questions about the properties and capabilities of cell membranes.

Biological membranes are heterogeneous, asymmetrical bilayers consisting mainly of ***lipids***, into which various ***membrane proteins*** (receptors, ion channels, enzymes, etc.) are embedded, whose functioning depends on their interaction with membrane lipids. Cholesterol plays a special role in this lipid regulation. In the presence of a specific genome and a corresponding set of proteins, this lipid component provides a wide range of regulation of cellular functions. In this book we will consider some examples of ***cholesterol-dependent membrane proteins*** and ***cellular processes*** and discuss their role in various microbial infections and other pathologies. In some of them, lowering cholesterol below the optimal physiological level can be detrimental to cells and be an important factor in pathogenesis.

Understanding the mechanisms of cholesterol interaction with proteins is a significant resource for the development of drugs that act at the protein–lipid interface and adjust these interactions without blocking cholesterol synthesis, the amount of which must remain sufficient, i.e., maintained at an optimal level.

The book is structured as follows. In **Chapter 1** we will briefly survey the membrane of an animal cell and the place of cholesterol in it, its quantity, sources and distribution, interactions with other membrane lipids, and its influence on the physical properties of membranes. **Chapter 2** goes over ***cholesterol-dependent membrane proteins*** and ***cholesterol-dependent processes***, as well as ***cholesterol-binding motifs in cholesterol-dependent proteins*.** We will look at some facts that demonstrate the importance of cholesterol for the normal functioning of membrane proteins and the entire cell. This lipid component ensures fine regulation of a whole range of cellular functions and provides the key to understanding changes in the activity of a number of proteins under various physiological and pathological conditions. The importance of cholesterol will be illustrated by examples of some cholesterol-dependent membrane proteins and cellular processes. Examples will be given of the influence of ***peptides with cholesterol-binding motifs*** on cholesterol-dependent processes as evidence in favour of the concept of cholesterol-binding motifs in cholesterol-dependent proteins. **Chapters 3** and **4** will discuss diseases in which cholesterol plays an important role in pathogenesis. **Chapter 3** provides examples of ***infectious diseases*** caused by pathogens that utilise host cell cholesterol and impair its homeostasis, while **Chapter 4** mentions some ***non-infectious diseases***, including not only and not so much atherosclerosis as diabetes, genetic and age-related diseases, in the pathogenesis of which cholesterol also plays a significant role. These diseases are caused by or associated with impairments in cholesterol synthesis and transport (enzymes, transporters, and

corresponding receptors), leading to lipid imbalance and cholesterol deficiency in cell membranes and corresponding cellular dysfunction.

The main goal and motivation of this book is an attempt to understand how we can (if necessary) help regulate cholesterol-dependent cellular processes in cells and how not to exacerbate problems associated with disturbances in this regulation. How much, where and when is cholesterol needed? What are its optimal concentrations in cells? What is the danger of ***losing membrane cholesterol*** and how can this be compensated for? To what extent do the levels of 'bad' and 'good' ***cholesterol in blood tests*** reflect the situation with ***cholesterol in cell membranes***? Should one go on a low-fat diet and take statins if blood tests show high levels of 'bad' cholesterol, or is it better to first understand the causes of lipid imbalance? It seems that a passive adherence to standard medical protocols without a basic knowledge of cell biology may violate the fundamental medical principle of 'do no harm.' Understanding the mechanisms of interaction between cholesterol and proteins can help to understand the pathogenesis of many diseases and become an important resource for the development of new therapeutic tactics and drugs that affect the interface between cholesterol and proteins and normalise these interactions.

The material presented in this book is intended for biologists and medical professionals, as well as readers who have a basic background in biology and organic chemistry and who are interested in and concerned with understanding how a healthy human body works, what happens during various diseases, how to help oneself in these situations, and what questions to ask a doctor. The references to peer-reviewed scientific articles are provided in the text and at the end of each chapter. These articles can help readers gain a deeper understanding of the scale of problems associated with cholesterol-dependent processes in cells, as well as the methods and results of

research in this field. Biology and, in particular, cell biology is still full of puzzles and mysteries that will have to be solved by future generations.

Chapter 1
Cholesterol in Cell Membranes

This chapter provides a brief description of biological membranes and their composition, focusing primarily on mammalian cell membranes, although all animals contain cholesterol in varying amounts. We will discuss the structure of the cell membrane (biomembrane) and its lipid composition; the cholesterol molecule, its content and distribution in the lipid bilayer of membranes, interactions with other membrane components and influence on the organisation and physical properties of membranes, as well as sources of cholesterol in cells, and where else, apart from membranes, it is used.

1.1. The lipid bilayer as the basis of the cell membrane

Every living cell, both prokaryotic and eukaryotic, is surrounded by a cell membrane (also called biological membrane, biomembrane), which provides both a diffusion barrier and regulation of incoming and outgoing flows of molecules across the cell boundary. There are many excellent reviews on this topic [1–5][1]. Cell membranes are also involved in a huge number of complex cellular processes, such as

[1] [1] Van Meer G., Voelker D.R., Feigenson G.W. 2008. Membrane lipids: Where they are and how they behave. *Nat. Rev. Mol. Cell Biol*. **9**, 112–124.
[2] Maxfield F.R., van Meer G. 2010. Cholesterol, the central lipid of mammalian cells. *Curr. Opin. Cell Biol*. **22** (4), 422–429.
[3] Ali O., Szabó A. 2023. Review of eukaryote cellular membrane lipid composition, with special attention to the fatty acids. Int. *J. Mol. Sci*. **24** (21), 15693.
[4] Song Y., Kenworthy A.K., Sanders C.R. 2014. Cholesterol as a co-solvent and a ligand for membrane proteins. *Protein Science*. **23**, 1–22.
[5] London E. 2019. Membrane structure-function insights from asymmetric lipid vesicles. *Acc. Chem. Res*. **52** (8), 2382-2391.

intra- and intercellular signalling, proliferation, differentiation, secretion, migration, endo- and phagocytosis, electrical and mechanical activity, and many others. About 5% of human genes are responsible for regulating the lipid composition of cell membranes [5].

Biological membranes are heterogeneous, asymmetrical ***bilayers*** consisting mainly of ***lipids***, to which more than half of all cellular ***proteins*** are permanently or temporarily bound – transmembrane, i.e. penetrating the lipid bilayer, and peripheral, i.e. bound only to the surface of the bilayer **(Fig. 1).**

A lipid bilayer is a double molecular layer formed by amphipathic lipids in an aqueous environment. Amphipathicity (or amphiphilicity) is the property of a molecule to possess both hydrophilic and lipophilic properties. Amphipathic molecules contain polar moieties that interact well with water and non-polar moieties that prefer to interact with fats or oils. Classic examples of amphiphilic substances are detergents, i.e. surfactants used in cleaning products. Surfactants bind hydrophobic groups of contaminants, 'shielding' them from polar water molecules and thus making them soluble in an aqueous environment. The lipids that make up cell membranes are also amphiphilic. They form bilayer structures (bilayers) in which the molecules are oriented so that their polar hydrophilic groups ('heads') are in contact with the aqueous environment, while the non-polar hydrophobic groups ('tails') face the inside of the bilayer and do not come into contact with water. The thickness of the lipid bilayer depends on the lipids that form it. A bilayer formed by 'cellular' lipids (*see below*) is approximately 4–5 nm thick.

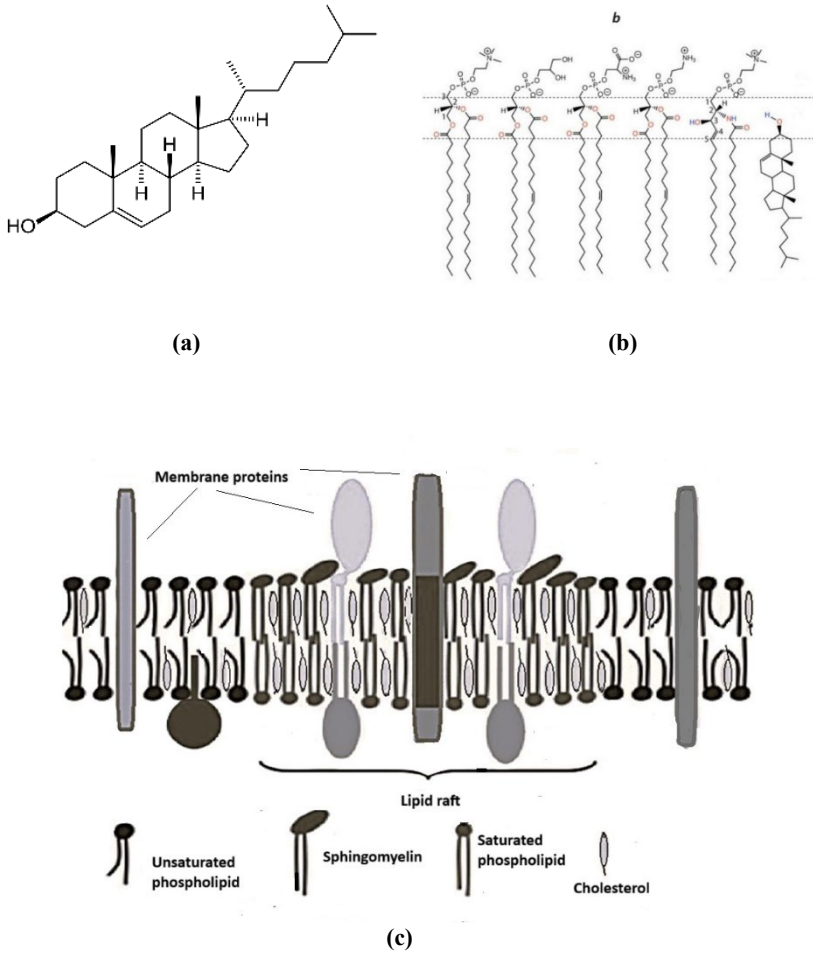

Fig. 1. Main components of the cell membrane. (a) Cholesterol molecule; (b) main lipids of the animal cell membrane: phospholipids and cholesterol; (c) diagram of the cell membrane: lipid bilayer with embedded proteins.

The diversity and organisation of membrane lipids play a crucial role in maintaining structural integrity, cellular homeostasis, and functional activity of cells, and changes in lipid composition are accompanied by changes in the state and activity of membrane

proteins, signalling pathways, and regulatory cascades. For experimental studies of lipid bilayers, artificial membranes, BLMs (bilayer lipid membranes) are made – both flat and in the form of vesicles – liposomes or nanosomes. Studies conducted on such artificial BLMs with a specified molecular composition help to understand the basic principles of biological membrane functioning, apply this knowledge in practical areas (e.g., the creation and use of liposomes for the delivery of biologically active substances) and raise new questions.

The main component (about 50 mol%) of the outer membranes surrounding the cell (called 'plasma membranes') are ***phospholipids***, which are complex esters of polyhydric alcohols and higher fatty acids and contain a phosphoric acid residue. Depending on the polyhydric alcohol in their composition, there are three groups of phospholipids: ***glycerophospholipids, phosphosphingolipids,*** and ***phosphoinositides.*** In ***glycerophospholipids***, the polyhydric alcohol is the trihydric alcohol ***glycerol***. This group of phospholipids includes phosphatidylcholine (PC, lecithin), phosphatidylethanolamine (PE, cephalin), phosphatidylserine (PS), cardiolipin (CL), and plasmalogen (ethanolamine plasmalogen). ***Phosphosphingolipids*** contain a sphingosine residue, an aliphatic amino alcohol with an unsaturated hydrocarbon chain (C18). This group of phospholipids includes sphingomyelins (SM). ***Phosphoinositides*** contain an inositol residue – a six-carbon cyclohexane alcohol; this group includes ***phosphatidylinositol*** (PI). The amphipathicity of phospholipids is provided by a combination of a hydrophilic 'head', a polar group of a phosphoric acid residue that comes into contact with the aqueous environment,

and hydrophobic 'tails', higher fatty acid residues that face the inside of the lipid bilayer [5–7][2].

Phospholipids are the main structural elements of the membrane and also play an important role in many cellular processes, such as the regulation of membrane proteins, membrane movement, cell growth, intracellular signalling, etc. In mammalian cells, phospholipids are formed as a result of several enzymatic steps, mainly in the endoplasmic reticulum, mitochondria, and Golgi apparatus. In recent years, various enzymes involved in the biosynthesis of phospholipid classes have been identified, and the relationship between abnormalities in phospholipid metabolism and various diseases is being investigated, as described in interesting reviews (e.g., Morita SY, Ikeda Y. 2022 [8][3]). Phospholipids are asymmetrically distributed across the bilayer of the plasma membrane. ***Phosphatidylserine*** (PS) and ***phosphatidylethanolamine*** (PE) are enriched in the ***inner leaflet*** of the lipid bilayer (i.e., in the lipid monolayer of the bilayer membrane that contacts the intracellular contents with its hydrophilic surface), while ***phosphatidylcholine*** (PC) and ***sphingomyelin*** (SM) are predominantly located in the ***outer leaflet***, with their hydrophilic heads facing the external environment.

Next in prevalence after phospholipids are ***sterols***, which are structurally very different from phospholipids. Sterols are polycyclic

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[2] [5] London E. 2019. Membrane structure-function insights from asymmetric lipid vesicles. *Acc. Chem. Res*. **52** (8), 2382-2391.
[6] Kamiya K., Kawano R., Osaki T., Akiyoshi K., Takeuchi S. 2016. Cell-sized asymmetric lipid vesicles facilitate the investigation of asymmetric membranes. *Nat. Chem*. **8**, 881–889.
[7] Kakuda S., Li B., London E. 2021. Preparation and utility of asymmetric lipid vesicles for studies of perfringolysin O-lipid interactions. *Meth. Enzymol*. **649**, 253–276.

[3] [8] Morita SY, Ikeda Y. 2022. Regulation of membrane phospholipid biosynthesis in mammalian cells. *Biochem Pharmacol.* **206**, 115296.

compounds chemically related to terpenes, found in large quantities in conifers and many essential oils. Sterols are the major non-polar lipids of cell membranes: cholesterol predominates in mammals, while ergosterol predominates in yeast, and phytosterols, in plants [1–4][4]. In the plasma membrane of vertebrates, the cholesterol content is about 40% (it can be lower or higher, up to 60%, *see below*), and in terms of its importance, it can be considered ***'the central lipid of mammalian cells'*** [2][5].

1.2. Cholesterol in cell membranes

A cholesterol molecule is a polycyclic amphipathic molecule formed on the basis of cyclopentaneperhydrophenanthrene **(Fig. 1).** Cyclopentanperhydrophenanthrene (also known as sterane or gonane) is a saturated tetracyclic hydrocarbon. Its various substituted derivatives are grouped under the general name of steroids. The sterane nucleus is the basis of the structure of many biologically and physiologically active substances, including not only cholesterol and other steroids (including steroid hormones), but also bile acids, cardiotonic glycosides, and other biologically important compounds.

Cholesterol contains a rigid part consisting of one five-membered and three six-membered rings, a conformational flexible 8-carbon chain in

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[4] [1] Van Meer G., Voelker D.R., Feigenson G.W. 2008. Membrane lipids: Where they are and how they behave. *Nat. Rev. Mol. Cell Biol*. **9,** 112–124.
[2] Maxfield F.R., van Meer G. 2010. Cholesterol, the central lipid of mammalian cells. *Curr. Opin. Cell Biol*. **22** (4), 422–429.
[3]. Ali O., Szabó A. 2023. Review of eukaryote cellular membrane lipid composition, with special attention to the fatty acids. *Int. J. Mol. Sci*. **24** (21), 15693.
[4] Song Y., Kenworthy A.K., Sanders C.R. 2014. Cholesterol as a co-solvent and a ligand for membrane proteins. Protein Science. 23, 1–22. doi 10.1002/pro.2385

[5] [2] Maxfield F.R., van Meer G. 2010. Cholesterol, the central lipid of mammalian cells. *Curr. Opin. Cell Biol*. **22** (4), 422–429.

the C-17 position, and a polar group ('head') formed by one hydroxyl group in the 3β position. This hydroxyl group is mainly located near the lipid-water interface and can form hydrogen bonds with the polar groups of membrane proteins or lipids. The rest of the cholesterol molecule is located between the hydrophobic chains of lipids (Fig. 1); according to Stek et al, 2024, the arrangement of cholesterol molecules in the plane between the monolayers of the lipid bilayer is also possible [9, 10][6].

The distribution of cholesterol between the lipid monolayers of the phospholipid bilayer is diverse and often asymmetrical, which can be explained by the different affinities of cholesterol for different phospholipids. In general, cholesterol distribution depends on phospholipids with anionic groups and saturated chains. The strongest interaction of cholesterol occurs with sphingolipids (glycosphingolipids and sphingomyelins). As noted in the previous section, phospholipids are asymmetrically distributed across the plasma membrane bilayer, which also affects the distribution of cholesterol in the bilayer. Phosphatidylserine (PS) and phosphatidylethanolamine (PE) are enriched in the inner leaflet of the plasma membrane, while phosphatidylcholine (PC) and sphingomyelin (SM) are predominantly located in the outer leaflet. Interactions of cholesterol with sphingomyelin and phosphatidylcholine, which are enriched with saturated chains, contribute to accumulation of cholesterol in the outer monolayer. The accumulation of cholesterol in the inner (cytoplasmic) lipid monolayer may be due to phosphatidylethanolamine and phosphatidylserine, whose content in this lipid

[6] [9] Steck T.L., Ali Tabei S.M., Lange Y. 2024. Estimating the cholesterol affinity of integral membrane proteins from experimental data. *Biochemistry*, **63** (1), 19–26.
[10] Steck T.L., Lange Y. 2018. Transverse distribution of plasma membrane bilayer cholesterol: Picking sides. *Traffic*. **19** (10), 750–760.

monolayer is elevated [8][7]. The trans-layer distribution of cholesterol is also significantly influenced by unsaturated fatty acids whose increased content in biomembranes increases the likelihood of cholesterol transition from one monolayer to another (flip-flop) [3, 9, 10][8].

Since cholesterol has different affinities for phospholipids and other membrane components, three operational pools of plasma membrane cholesterol are distinguished, depending on its mobility and accessibility to various molecular probes (and therefore to various biologically significant substances) [9, 10][9]. The most mobile pool is cholesterol ('active cholesterol'), which is recognised by the D4 domain of perfringolysin O (PFO) and anthrolysin O (ALOD4). This pool of active cholesterol accounts for approximately 10 mol% of plasma membrane lipids and becomes unavailable when the cholesterol content in plasma membrane decreases and becomes less than 30 mol%; this pool quickly moves to the ER, where the signal of cholesterol deficiency is perceived by the SREBP2 mechanism[10] [11,

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[7] [8] Morita SY, Ikeda Y. 2022. Regulation of membrane phospholipid biosynthesis in mammalian cells. *Biochem Pharmacol*. **206**, 115296.

[8] [3] Ali O., Szabó A. 2023. Review of eukaryote cellular membrane lipid composition, with special attention to the fatty acids. *Int. J. Mol. Sci*. **24** (21), 15693.

[9] Steck T.L., Ali Tabei S.M., Lange Y. 2024. Estimating the cholesterol affinity of integral membrane proteins from experimental data. *Biochemistry*, **63** (1), 19–26.

[10] Steck T.L., Lange Y. 2018. Transverse distribution of plasma membrane bilayer cholesterol: Picking sides. *Traffic*. **19** (10), 750–760.

[9] [9] Steck T.L., Ali Tabei S.M., Lange Y. 2024. Estimating the cholesterol affinity of integral membrane proteins from experimental data. *Biochemistry*, **63** (1), 19–26.

[10] Steck T.L., Lange Y. 2018. Transverse distribution of plasma membrane bilayer cholesterol: Picking sides. *Traffic.* **19** (10), 750–760.

[10] [11] Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, Goldstein JL. 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA.* **100** (21), 12027-12032.

12]. The second pool of cholesterol, recognised by ostrholeysin A (OlyA), is part of sphingomyelin/cholesterol complexes. This pool of cholesterol accounts for about 15 mol% of PM lipids and forms the basis of rafts. The rest of the cholesterol (about 15 mol% of PM lipids) is sequestered by other membrane factors and is critical for cell viability; currently, there are no probes for this cholesterol pool.

It is interesting and biologically very important that the presence of cholesterol in the bilayer lipid membrane affects the behaviour of phospholipids, in particular, the rate of their trans-bilayer lipid movement (flip-flops), i.e., the transition of phospholipid molecules from one monolayer to another. While cholesterol itself is capable of rapidly performing such transitions at room temperature or 37°C, cholesterol slows down both spontaneous and peptide-enhanced flip transitions of phospholipids [13, 14]. Nakano et al., 2009 [13][11] showed that cholesterol completely inhibited the flip-flop movement of dimyristoylphosphatidylcholine (DMPC) and 1-palmitoyl-2-oleoyl-phosphatidylcholine (or 1-palmitoyl-2-oleoylphosphatidic acid (POPA). Based on their data, the authors suggested that in cell plasma membranes rich in cholesterol, the flip-flop of phosphatidylcholines does not occur spontaneously and requires the enzymatic activity of energy-dependent and/or energy-independent flipase/floppase enzymes. Similar results were obtained in the work of LeBarron and

[12] Lebeau PF, Byun JH, Platko K, Saliba P, Sguazzin M, MacDonald ME, Paré G, Steinberg GR, Janssen LJ, Igdoura SA, Tarnopolsky MA, Wayne Chen SR, Seidah NG, Magolan J, Austin RC. 2022. Caffeine blocks SREBP2-induced hepatic PCSK9 expression to enhance LDLR-mediated cholesterol clearance. *Nat. Commun*. **13** (1), 770.

[11] [13] Nakano M; Fukuda M; Kudo T; Matsuzaki N; Azuma T; Sekine K; Endo H; Handa T. 2009. Flip-flop of phospholipids in vesicles: kinetic analysis with time-resolved small-angle neutron scattering. *J. Phys. Chem. B.* **113**, 6745–6748.

London [14][12]: accelerated flip-flop was slowed down in lipid vesicles containing 30% cholesterol. This means that the lower the cholesterol content in the membrane, the easier it is to perform such flip-flops, i.e., the easier it is to lose asymmetry in the trans-bilayer distribution of phospholipids. Looking ahead, it can be assumed that the loss of cholesterol from the plasma membrane increases the flip-flop rate of phosphatidylserine (PS), which is mainly located in the inner lipid monolayer. In cells with cholesterol deficiency, PS will move to the outer lipid monolayer, which is a 'eat me' signal for macrophages [15][13] because the loss of cholesterol means cell damage, and macrophages must phagocytose such cells.

In addition to influencing the flip-flop movement of phospholipids, cholesterol affects the structure and physical properties of biological membranes [1–5][14]. By filling the spaces between phospholipids in the bilayer of the membrane, cholesterol has a significant effect on membrane parameters such as thickness, rigidity, elasticity, fluidity, thermosensitivity, permeability, membrane protein mobility, and

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[12] [14] LeBarron J, London E. 2016. Effect of lipid composition and amino acid sequence upon transmembrane peptide-accelerated lipid transleaflet diffusion (flip-flop). *Biochim. Biophys. Acta*. **1858**, 1812–1820.

[13] [15] Flannagan RS, Canton J, Furuya W, Glogauer M, Grinstein S. 2014. The phosphatidylserine receptor TIM4 utilises integrins as coreceptors to effect phagocytosis. *Mol Biol Cell*. **25** (9), 1511-1522.

[14] [1] Van Meer G., Voelker D.R., Feigenson G.W. 2008. Membrane lipids: Where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* **9**, 112–124.
[2] Maxfield F.R., van Meer G. 2010. Cholesterol, the central lipid of mammalian cells. *Curr. Opin. Cell Biol*. **22** (4), 422–429.
[3] Ali O., Szabó A. 2023. Review of eukaryote cellular membrane lipid composition, with special attention to the fatty acids. *Int. J. Mol. Sci*. **24** (21), 15693.
[4] Song Y., Kenworthy A.K., Sanders C.R. 2014. Cholesterol as a co-solvent and a ligand for membrane proteins. *Protein Science*. **23**, 1–22.
[5] London E. 2019. Membrane structure-function insights from asymmetric lipid vesicles. *Acc. Chem. Res*. **52** (8), 2382-2391.

essentially determines the lateral organisation of the membrane [16–19][15]. Cholesterol reduces membrane fluidity, increases membrane thickness and mechanical stability, and reduces membrane permeability. One of the parameters used to assess membrane stiffness is the phospholipid/cholesterol ratio.

An important and perhaps unexpected property of cholesterol is its effect on the permeability of membranes to gases, especially carbon dioxide, CO_2 (see review by Endeward et al, 2014 [20][16]). Cholesterol can 'adjust' the permeability of the membrane to CO_2 depending on the functional needs of the cell: the higher the concentration of cholesterol in the membrane, the lower its permeability to CO_2. Experiments conducted by Itel et al. 2012 [21][17] showed that reducing the cholesterol content in MDCK cells by removing it with β-cyclodextrin led to an increase in permeability to CO_2 by more than 40 times. Conversely, enriching the membranes of these MDCK cells with β-cholesterol reduced PCO_2 to 1/3 of the normal level. The results of molecular dynamics modelling by Hub et al. 2010 [22][18] predict that

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[15][16] Simons K., Ikonen E. 1997. Functional rafts in cell membranes. *Nature*. **387**, 569–572.

[17] Genova J., Bivas I., Marinov R. 2014. Cholesterol influence on the bending elasticity of lipid membranes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. **460**, 79-82.

[18] Pike L.J. 2003. Lipid rafts: Bringing order to chaos. *J. Lipid Res*. **44** (4), 655–667.

[19] Helms J.B., Zurzolo C. 2004. Lipids as targeting signals: Lipid rafts and intracellular trafficking. *Traffic*. **5** (4), 247–254.

[16] [20] Endeward V, Al-Samir S, Itel F, Gros G. 2014. How does carbon dioxide permeate cell membranes? A discussion of concepts, results and methods. *Front Physiol*. **4**, 382.

[17] [21] Itel F., Al-Samir S., Öberg F., Chami M., Kumar M., Supuran C. T., et al. 2012. CO_2 permeability of cell membranes is regulated by membrane cholesterol and protein gas channels. *FASEB J.* **26**, 5182–5191.

[18] [22] Hub J.S., Winkler F.K., Merrick M., de Groot B.L. 2010. Potentials of mean force and permeabilities for carbon dioxide, ammonia, and water flux across a Rhesus protein channel and lipid membranes. *J. Am. Chem. Soc*. **132**, 13251–13263.

cholesterol can reduce the permeability of lipid bilayers to CO_2 by several orders of magnitude. Düttingdorf et al., 1999 [23][19] found that the cholesterol content in the apical and basolateral membranes of the guinea pig colon epithelium differs significantly: the cholesterol content in the basolateral membrane of the proximal colon was 42%, which corresponds to the content in many cells, but in the apical membrane, the cholesterol content reaches 77%, which satisfactorily explains its low permeability to CO_2. Thus, cells can regulate their permeability to CO_2 (and probably also NH_3) over a wide range simply by changing the cholesterol content in their membrane.

As mentioned above, cholesterol plays an important role in the lateral organisation of the membrane. Due to the special affinity of cholesterol for sphingomyelin, which has well-ordered and tightly packed acyl chains, these two lipids can aggregate and form liquid-ordered and detergent-resistant microdomains called ***lipid rafts*** [5, 16–18, 24–26][20].

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[19] [23] Düttingdorf H.-D, Sallmann H.-P., Glockenthör U, Engelhardt W, Busche R. 1999. Isolation and lipid composition of apical and basolateral membranes of colonic segments of guinea pig. *Analytical Biochem*. **269**, 45-53.

[20] [5] London E. 2019. Membrane structure-function insights from asymmetric lipid vesicles. *Acc. Chem. Res*. **52** (8), 2382-2391.

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[17] Genova J., Bivas I., Marinov R. 2014. Cholesterol influence on the bending elasticity of lipid membranes, *Colloids and Surfaces A: Physicochem. Engineer. Aspects.* **460**. 79-82.

[18] Pike L.J. 2003. Lipid rafts: Bringing order to chaos. *J. Lipid Res*. **44** (4), 655–667.

[24] Schroeder R., London E., Brown D. 1994. Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behaviour. *Proc. Natl. Acad. Sci. USA*. **91**, 12130–12134.

[25] Brown D.A., London E. 1997. Structure of detergent-resistant membrane domains: Does phase separation occur in biological membranes? *Biochem. Biophys. Res. Comm*. **240**, 1–7.

[26] Ahmed S.N., Brown D.A., London E. 1997. On the origin of sphingolipid/ cholesterol-rich detergent-insoluble cell membranes: Physiological concentrations

A working model of the origin of sphingolipid- and cholesterol-rich rafts in biological membranes was proposed by the Erwin London's group in collaboration with Dr. Deborah Brown's group [24–27][21]. Sphingolipids typically have acyl chains without double bonds and a high melting temperature (Tm), while most phospholipids have at least one unsaturated acyl chain and a low Tm. In biomembranes (e.g., plasma membranes) containing a mixture of both classes of lipids, gel (gel-like, more 'solid', ordered) domains and liquid disordered (Ld) domains can coexist**.** In the presence of cholesterol, an intermediate state may form, a liquid ordered state (Lo). In eukaryotes, such Lo microdomains, or ***rafts***, are characterised by a denser packing of membrane lipids and a higher degree of order than in the surrounding liquid disordered bilayer, as well as resistance to detergents, rapid lateral diffusion and a higher concentration of cholesterol and sphingolipids. The size, stability, and distribution of rafts, as in the case of transbilayer cholesterol distribution, are regulated by other

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of cholesterol and sphingolipid induce formation of a detergent-insoluble, liquid-ordered lipid phase in model membranes. *Biochemistry.* **36**, 10944–10953.

[27] Toledo A., Huang Zh., Coleman J.L., London E., Benach J.L. 2018. Lipid rafts can form in the inner and outer membranes of *Borrelia burgdorferi* and have different properties and associated proteins. *Mol. Microbiol*. **108** (1), 63–76.

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membrane components, in particular unsaturated fatty acids, which have a lower affinity for cholesterol than their saturated counterparts. Recent studies by E. London, 2019 [4, 35, 36][22] showed that trans-bilayer asymmetry of membranes affects lipid movement across the bilayer and the formation of ordered lipid domains, and that there is a link between the physical properties in each monolayer and the conformation of membrane proteins.

Many works have been written about the numerous functions of rafts, in which certain membrane proteins are assembled/concentrated /accumulated [16, 18, 19, 27–29][23]. These microdomains not only affect the lateral mobility of the membrane, but also actively participate in various cellular events, in particular, due to their association with certain proteins that perform important biological functions [29][24]. The accumulation/concentration of such membrane proteins in these lipid microdomains in itself affects their activity, since the functioning of membrane proteins depends on their lipid environment (this will be discussed in Chapter 2). In addition, the close proximity of certain proteins within a lipid domain can optimise their interaction, which is necessary for cell signalling, metabolism and other processes. Overall, rafts—lipid domains in membranes enriched with cholesterol and sphingomyelin—are a necessary component of cell membranes, participating in the organisation of cell receptor and signalling systems and in ensuring/maintaining cell homeostasis.

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[23] [16, 18, 19, 27–29]
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1.3. Distribution of cholesterol in cell compartments and intracellular transport of cholesterol

Cholesterol is not only a component of plasma (outer) cell membranes, but also an important component of the membranes of intracellular organelles – the nucleus, mitochondria, endoplasmic reticulum (ER), mitochondria, endosomes and phagosomes, lysosomes, etc., where biomembranes ensure the coordination of various functions within the cell and organelles, control the movement of macro- and micromolecules, and create surfaces on which important biological events take place. The distribution of cholesterol (as well as other lipids) in these cellular compartments (inside cells between different cell membranes) is very uneven/heterogeneous. As already noted, the cholesterol content in the plasma membrane is 30–50% of the total amount of lipids, which is the highest concentration of cholesterol compared to other cell membranes. The ER is poor in cholesterol and contains 3–6% of the total lipids of the ER membrane (this is less than 1% of the total cholesterol in the cell) (see detailed reviews [30, 31][25]. **Table 1** illustrates the differences in the lipid composition of the plasma membrane and the membrane of the endoplasmic reticulum (ER) of an animal cell [30][26]. Membranes communicating with the plasma membrane, such as the trans-Golgi network and recirculating endosomal compartments, contain intermediate amounts of cholesterol. Towards the cis-Golgi and the nucleus, the cholesterol

[25] [30] Ikonen E., Zhou X. 2021. Cholesterol transport between cellular membranes: A balancing act between interconnected lipid fluxes. *Dev. Cell*. **56** (10), 1430-1436.
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[26] [30] Ikonen E., Zhou X. 2021. Cholesterol transport between cellular membranes: A balancing act between interconnected lipid fluxes. *Dev. Cell*. **56** (10), 1430-1436.

content decreases further, although the nuclear envelope contains 'raft' lipids sphingomyelin and cholesterol, as well as microdomains enriched with these lipids [32–34][27]. It should be noted that nuclear lipids and the signalling functions of nuclear lipids, in particular cholesterol, are attracting increasing attention, as studies show that cholesterol and other lipids that make up the nuclear envelope and chromatin are active participants in intranuclear processes and play an active role in the implementation and regulation of cell division and differentiation processes and in apoptosis.

Table 1. Distribution of phospholipids and cholesterol in the plasma membrane (PM) and endoplasmic reticulum (ER) membrane (based on [30][28])

Lipid type	% of all lipids in this type of membrane	
	PM	ER
Phospholipids	~51–56	~82
Phosphatidylcholine (PC)	~25	~60
Phosphatidylserine (PS)	~2	<2
Phosphatidylinositol (PI)	~2	≥5
Phosphatidylethanolamine (PE)	~12	~20
Sphingomyelin (SM)	~10–15	~0
Cholesterol	~40	≤5%
Other lipids	~4–9	~13

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[28] [30] Ikonen E, Zhou X. 2021. Cholesterol transport between cellular membranes: A balancing act between interconnected lipid fluxes. *Dev. Cell*. **56** (10), 1430-1436.

The low cholesterol content of intracellular membranes makes them very sensitive to changes in cholesterol levels. Due to the maintenance of a very low initial cholesterol level in the endoplasmic reticulum, even small changes in cholesterol levels in the plasma membrane lead to a significant (an order of magnitude) jump in cholesterol content in the endoplasmic reticulum [30, 35][29]. Although the biosynthesis of most classes of membrane lipids (including cholesterol, glycerophospholipids, and sphingolipids), mitochondria and the Golgi apparatus are involved in addition to the endoplasmic reticulum, most of the enzymes involved in cholesterol synthesis, as well as the main part of the molecular mechanism regulating its cellular homeostasis (the sterol regulatory element binding protein/SREBP cleavage activating protein system, SREBP/SCAP) is located precisely in the membranes of the endoplasmic reticulum [36–38][30]. From there, cholesterol is rapidly transported to other cell membranes.

Cholesterol is not only synthesized in the ER, but also comes from the extracellular environment, where it is transported in the form of

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[29] [30] Ikonen E, Zhou X. Cholesterol transport between cellular membranes: A balancing act between interconnected lipid fluxes. *Dev. Cell*. **56** (10), 1430-1436.
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lipoprotein particles – low-density lipoproteins (LDL). LDL binds to LDL receptors on the surface of the PM and enters the cell by receptor-mediated endocytosis. LDL is then delivered to lysosomes, where cholesterol is released from LDL and transported to the PM. After the PM is enriched with cholesterol, it is exported to the ER. When the cholesterol content in the ER exceeds ~5% of the total ER lipid mass, cholesterol synthesis and LDL receptor production in the ER are down-regulated. In addition, excess cholesterol in the ER is esterified to cholesterol esters for storage in fat droplets. Such strict regulation of cholesterol homeostasis and distribution is essential for normal cell viability and growth [38][31].

Cholesterol homeostasis and distribution in the cell are strictly regulated, and the cell uses various systems of intermembrane cholesterol transport (as this is essential for normal cell viability and growth). The review by Ikonen and Zhou [30][32] describes the main intracellular pathways of cholesterol transport and the main points of their intersection. Cholesterol in the cell is rarely transported alone and is usually combined with the transport and metabolism of other lipids, in particular phosphoinositides, phosphatidylserine and sphingolipids. In addition to exo- and endocytosis mechanisms, lipid transport proteins are used to transport cholesterol, whose hydrophobic cavities protect the lipid from water and can catalyse the transfer of lipids between organelles. The transport of cholesterol from the PM to the ER in mammals requires the anionic phospholipid phosphatidylserine, as well as ER- and PM-associated Aster proteins. Intracellular membrane contacts ***(membrane contact sites*, MCS)** are

[31] [38] Goldstein and Brown
[32] [30] Ikonen and Zhou

involved in cholesterol transport processes. These are areas where the distance between membranes is about 10 nm [30, 35, 40][33].

The molecular structure and role of such contacts in cellular processes and metabolic rearrangements of cells is of great interest and is becoming an important topic in cell biology. Although this is a relatively young field of research, it is already clear that MCSs are involved in lipid transport and lipid signalling, and also play an important role in calcium signalling and in the adaptation of cells to stress. New tools for visualising and studying MCS have shown that MCS are ubiquitous and function as signalling, metabolic, and logistical centres that coordinate the work of organelles in both normal physiological and stressful conditions (see review [41][34]). Intermembrane cholesterol transport, carried out with the participation of MCS, appears to be one of the essential factors in this coordination.

Among ***membrane contact sites*** (MCS), the contacts between the endoplasmic reticulum and mitochondria (EMCS) are particularly noteworthy, as they are considered central hubs for lipid transport between these organelles and key effectors of non-vesicular lipid trafficking in mammalian cells. EMCS regulate the lipid composition of cell membranes and organelles, their physiological functions, and lipid-mediated signalling pathways in both physiological and pathological conditions. A review by Sassano et al. 2022 [42][35]

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[33] [30] Ikonen and Zhou, [35] Radhakrishnan et al., 2008
[40] Kennelly JP, Tontonoz P. 2023. Cholesterol transport to the endoplasmic reticulum. *Cold Spring Harb Perspect Biol*. **15** (2), a041263.

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[35] [42] Sassano ML, Felipe-Abrio B, Agostinis P. 2022. ER-mitochondria contact sites; a multifaceted factory for Ca^{2+} signalling and lipid transport. *Front. Cell Dev. Biol*. **10**, 988014.

discusses key aspects of the functional complexity of EMCS in mammalian cells, as well as how disruptions in these pathways may contribute to the development of key hallmarks of cancer cells.

Overall, the distribution and maintenance of physiological cholesterol levels in cells is achieved through a variety of mechanisms, indicating that this is a vital task for the cell. Cholesterol is essential for animal cells, and its deficiency or excess is destructive to cells. Therefore, complex molecular mechanisms have evolved to maintain optimal levels of this sterol, controlling and regulating the levels of cholesterol and other related sterols, such as oxysterols or intermediate products of cholesterol synthesis, and responding to changes in their levels through various regulatory feedback mechanisms. This regulation includes both direct binding of sterols by components of the homeostatic system located in the ER and indirect effects caused by cholesterol-dependent changes in the physical properties of membranes.

1.4. Sources of cholesterol in animal cells

The ability of cells to survive and function depends on strict control of free cholesterol levels in cells. A delicate balance between synthesis, uptake, modification, and excretion allows cells to maintain proper cholesterol distribution among organelles (see [40][36]). Most animal cells possess the necessary enzymes and mechanisms for cholesterol synthesis. ***Endogenous cholesterol*** is synthesised from acetyl-CoA (see diagram in Fig. 2), and all carbon atoms in cholesterol originate from acetate. Currently, the following stages are distinguished in the cholesterol biosynthesis pathway: (1) Conversion of three molecules of active acetate into five-carbon ***mevalonate*** (three reactions, the first

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