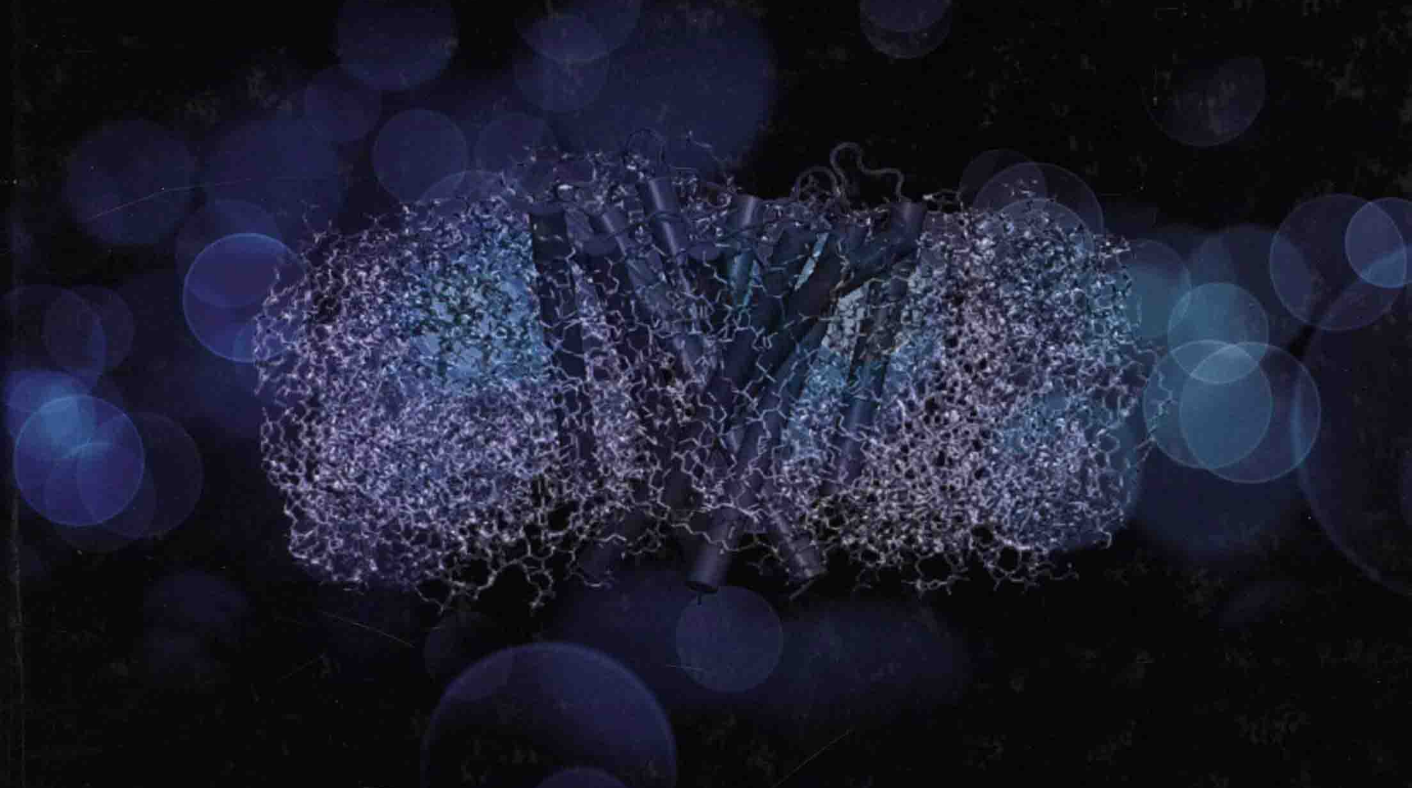
An Introduction To

Biological Membranes

FROM BILAYERS TO RAFTS



William Stillwell

AN INTRODUCTION TO BIOLOGICAL MEMBRANES
From Bilayers to Rafts

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AN INTRODUCTION TO BIOLOGICAL MEMBRANES

From Bilayers to Rafts

Dedication

This book is dedicated to my wife Penelope who has tolerated me all these years, my son Max, my daughter Jessica, and Cosmo, the brains of the operation.

Preface

Virtually all biological processes are in some fashion involved with membranes. However, despite their unquestioned importance, many aspects of complex membrane structure and function remain unresolved. Often not only are the answers not available, but even formulating the appropriate questions is difficult. A single membrane is composed of hundreds of proteins and thousands of lipids, all in constant flux. Methodologies to study membranes must span time and size domains that range over a million fold! A good case can be made that understanding membranes will be the next great frontier in the life sciences.

From the inception, it became obvious to me that trying to write a book on membranes is a near hopeless endeavor. No-one can possibly be an expert in all aspects of membrane science. Membrane studies range from structural and theoretical biophysics at one extreme to nutritional, cell biology, and membrane metabolic studies at the other. Bridging the two extremes are countless highly technical methodologies that are difficult to explain without getting hopelessly bogged down in technical minutia. Choosing what should be included and what excluded from this book reflects my personal 40 years of research experience on the biophysics and biochemistry of model membrane systems.

Through the years there have been a large number of books written about membranes. Most of these have been multi-authored, highly technical compendiums that are essentially "preaching to the choir". They are indecipherable to anyone who is not already an expert in the narrow topic covered in the book. While these multi-authored books have their place in membrane studies, they are essentially a non-cohesive collection of loosely related review articles that are written by experts from very different backgrounds and writing styles. As a result they are comprehensive, but very hard to read. In sharp contrast, excellent general discussions covering membranes can now be found in very well written chapters in almost all current biochemistry, cell and molecular biology, and physiology textbooks. These brief chapters are of necessity too abbreviated to adequately cover a field as expansive as membranes. The multi-authored compendiums are too detailed while the chapters from general textbooks lack sufficient detail.

Between the extremes of edited compendiums and general textbook chapters are a smaller number of general membrane books that have the advantage of being written by a single (or at most a few) authors. *An Introduction to Biological Membranes: From Bilayers to Rafts* is such a book. This book is an attempt to write a broad textbook covering many aspects of membrane structure/function that bridges membrane biophysics and cell biology. It was my basic contention that it is not necessary to understand the intricacies of NMR, single particle tracking, X-ray crystallography and so on, to appreciate the contributions that these techniques have made to membrane studies. The book primarily targets advanced undergraduates and beginning graduate students. However, even membranologists with a good knowledge of contributions by their contemporaries often have very limited knowledge of fundamental contributions by early membrane pioneers, a recurring theme of this book. The appendix to this book is a time line of 100 of the most important discoveries in membrane science dating from ~540 BC to present.

In the process of cleaning out my office before fading into retirement in 2010, I found that I had accumulated about 60 membrane books, and this is only a fraction of what is available. For 30 years I taught a beginning graduate level course entitled *Biological Membranes*. For most of those years I could not find a suitable textbook for the course. In 1988 Robert B. Gennis published his book *Biomembranes: Molecular Structure and Function*. To this day I consider Gennis' book to be the best membrane structure book ever written and for about 10 years I adopted this book for my course. Unfortunately, after 25 years, a second edition of this book has yet to be published. Something is needed to fill this gap. From my perspective, the new book should be a single-authored, broad book that covers many aspects of membrane structure and function without getting lost in unnecessary details – in other words, 'membrane biophysics light'. It is clear that each topic covered in *An Introduction to Biological Membranes: From Bilayers to Rafts* could be greatly expanded, but this will have to wait for another day.

Through the years other single-authored general membrane books have appeared, each of which has its advantages and disadvantages. A few examples are listed below:

Gennis, R.B. 1988. Biomembranes: Molecular Structure and Function. Springer-Verlag. New York, NY. 533 pp.

Luckey, M. 2008. Membrane Structural Biology. With Biochemical and Biophysical Foundations. Cambridge University Press, New York, NY, 332 pp.

Mouritsen, O.G. 2005. Life as a Matter of Fat. The Emerging Science of Lipidomics. Springer, Berlin, Heidelberg, 276 pp.

Jones, M.N. and Chapman, D. 1995. Micelles, Monolayers and Biomembranes. John Wiley and Sons, New York. 252 pp.

Yeagle, P.L. 1993. The Membranes of Cells, 2nd Edition. Academic Press, New York. 349 pp.

Jain, M.K. 1988. Introduction to Biological Membranes, 2nd Edition. John Wiley & Sons, New York. 423 pp.

Finean, J.B., Coleman, R. and Michell, R.H. 1984. Membranes and Their Cellular Functions, 3rd Ed. Blackwell Scientific Publications, Oxford, New York. 227 pp.

Malhotra, S.K. 1983. The Plasma Membrane. John Wiley and Sons, New York. 209 pp.

Houslay, M.D. and Stanley, K.K. 1982. Dynamics of Biological Membranes: Influence on Synthesis, Structure and Function. John Wiley & Sons, New York. 330 pp.

William Stillwell
Sand Key, Florida
January, 2013

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CHAPTER

1

Introduction to Biological Membranes

OUTLINE

A. WHAT IS A BIOLOGICAL MEMBRANE?

The American Heritage Dictionary defines a membrane as 'a thin pliable layer of plant or animal tissue covering or separating structures or organs.' The impression this description leaves is one of the plastic wrap covering a hamburger. By this definition, membranes are static, tough, impenetrable, and visible. Yet, nothing could be farther from the truth. The entire concept of *dynamic* behavior is missing from this definition, yet dynamics is what makes membranes both essential for life and so difficult to study.

If we could somehow instantaneously freeze a membrane and learn the composition and location of each of the countless numbers of molecules comprising the membrane, and then instantly return the membrane back to its original unfrozen state for a microsecond before re-freezing, we would find that the membrane had substantially changed while unfrozen. Although the molecular composition would remain the same over this short time, the

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molecular locations and interrelationships would be altered. Therefore membranes must have both static and dynamic components. While static describes what is there, dynamics describes how the components interact to generate biological function.

Every cell in the human body is a tightly packed package of countless membranes. The human body is composed of ~63 trillion cells (6.3×10^{13} cells), each of which is very small. For example, a typical liver cell would have to be 5X larger to be seen as a speck by someone with excellent vision (it is microscopic). Each liver cell has countless numbers of internal membranes. If you could somehow open one single liver cell and remove all of the internal membranes and sew them together into a quilt, the quilt would cover ~840 acres, the size of New York's Central Park! And that is from one single cell. Therefore, there are enough membranes in a human body (6.3×10^{13} cells) to cover the earth millions of times over!

All life on Earth is far more similar than it is different. Living organisms share a number of essential biochemical properties, collectively termed the 'thread of life'. Included in these essential properties is ownership of a surrounding plasma membrane that separates the cell's interior from its external environment. It is likely that all living things inhabiting planet Earth today arose from a single common ancestor more than 3.5 billion years ago. The first cell probably contained minimally a primitive catalyst (a pre-protein), a primitive information storage system (a pre-nucleic acid), a source of carbon (perhaps a primitive carbohydrate) and this mixture had to be surrounded by a primitive plasma membrane that was likely made of polar lipids. Membranes were therefore an essential component of every cell that is alive today or has ever been alive.

With 3.5 billion years of biological evolution, the complexity of membranes in cells has greatly expanded from that of a simple surrounding plasma membrane to where they now occupy a large portion of a eukaryote's interior space. An electron microscopic picture of a 'typical' eukaryotic (liver) cell is shown in Figure 1.1 [1]. It is evident from the complexity of this micrograph that identifying, isolating, and studying membranes will be a difficult task.

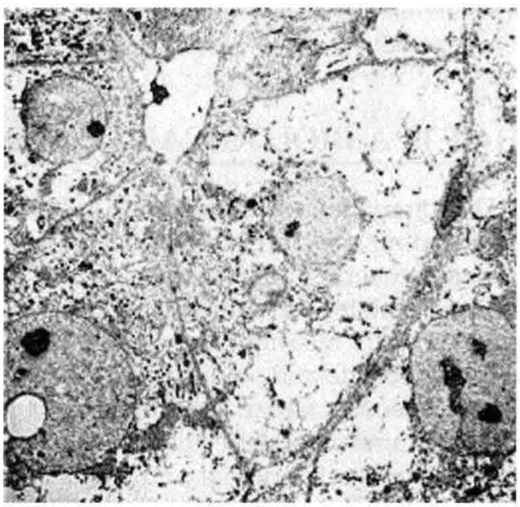

FIGURE 1.1 Transmission electron micrograph of a liver cell, a 'typical' cell [1].

B. GENERAL MEMBRANE FUNCTIONS

It is now generally agreed that biological membranes are probably somehow involved in all cellular activities. The most obvious function of any membrane is separating two aqueous compartments. For the plasma membrane this involves separation of the cell contents from the very different extra-cellular environment. Membranes are therefore responsible for containment, ultimately delineating the cell. Separation, however, cannot be absolute, as the cell must be able to take up essential nutrients, gases, and solutes from the exterior, while simultaneously removing toxic waste products from the interior. A biological membrane therefore must be selectively permeable, possessing the ability to distinguish many chemically different solutes and knowing in which direction to redistribute them. Biological membranes must therefore house a variety of specific, vectorial transport systems (discussed in Chapter 14).

A characteristic of all living cells is the establishment and maintenance of trans-membrane gradients of all solutes. Of particular interest are large ion gradients typically associated with the plasma membrane. Table 1.1 is a comparison of the mean concentration of selected ions inside and outside a typical mammalian cell, and the magnitude of each gradient. To maintain gradients of this size, efficient energy-dependent transport systems must be employed (discussed in Chapter 14). Directional trans-membrane structure is required to generate these ion gradients.

In addition to trans-membrane structure, it is now believed that biological membranes are composed of countless numbers of very small, transient, lateral lipid microdomains. Each of these domains is proposed to have a different lipid and resident protein composition. Thus the activity of any membrane must reflect the sum of the activities of its many specific domains. One type of lipid microdomain, termed a 'lipid raft', has received a lot of recent attention as it is reputed to be involved in a variety of important cell signaling events. If the lipid raft story (discussed in Chapter 8) holds up, this new paradigm for membrane structure/function may serve as a model for other types of as yet undiscovered non-raft domains. Each of these domains might then support a different collection of related biochemical activities. Therefore, membranes have both trans-membrane and lateral structures that are just beginning to be understood.

All membranes possess an extreme water gradient across their very thin (~5 nm) structure. In the membrane aqueous bathing solution water concentration is ~55.5 M water in water (1,000 g of water per liter divided by 18, the molecular weight of water), while the membrane

TABLE 1.1 Trans-membrane Ion Gradients of a 'Typical' Mammalian Cell.

Ion	Inside	Outside	Gradient
Na^+	10 mM	140 mM	14-fold
K^+	140 mM	4 mM	35-fold
Ca^{2+}	1.0 μM	1.0 mM	1,000-fold
Cl^-	100 mM	4.0 mM	25-fold

interior is quite dry (< 1 mM water). The aqueous interface provides a charged or polar physical surface to help arrange related functional enzymes, known as pathways, in one plane for increased efficiency. In contrast, the dry interior provides an environment for dehydration reactions. Both the aqueous interface and dry interior are responsible for maintaining the proper conformation of membrane proteins (discussed in Chapter 6).

In addition to transport, biological membranes are also the site of many other biochemical or physiological processes including: inter-cellular communication, cell–cell recognition and adhesion, cell identity and antigenicity, regulation (resident home of many receptors), intracellular signaling, and some energy transduction events.

C. EUKARYOTE CELL STRUCTURE

While the plasma (cell) membrane defines cell boundaries, internal membranes define a variety of cell organelles. In eukaryotes, the internal membranes also separate very different internal aqueous chambers resulting in compartmentation into membrane-bordered packets called organelles. Each organelle supports different sets of biological functions. Figure 1.2 is a cartoon depiction of a 'typical' animal (eukaryote) cell [2]. All of the important membrane-bound organelles are depicted. Throughout this book, specific examples demonstrating aspects of membrane structure or function will be selected from these organelle types. Below is a very brief description of the major cellular membranes. More detailed descriptions can be readily found in many cell biology [3–6], and biochemistry [7,8] textbooks.

Endomembrane System

With the exception of mitochondria, peroxisomes and, in plants, chloroplasts, the intracellular membranes are suspended together in the cytoplasm where they form an interconnected complex. Although membrane types are unique and can be separated from one another, all are related structurally, chemically, functionally, and developmentally. This strong inter-relationship is referred to as the 'endomembrane system'. The endomembrane system divides the cell into many compartments, primarily organelles, which are distinct yet interconnected. The connected membranes include the nuclear envelope, the rough and smooth endoplasmic reticulum, the Golgi apparatus, lysosomes, vacuoles, many types of small vesicles, and the plasma membrane (Figure 1.3) [9]. Transport back and forth between these various membranes and compartments is continuous and under strict regulation [10]. The endomembrane system is the foundation of cell trafficking.

Plasma Membrane

All cells are surrounded by a plasma membrane (PM) that separates the cell contents from the rest of the world [11]. The PM is the most dynamic and busiest of all cellular membranes and more is known about the PM than any other membrane. The majority of examples described in this book were obtained from PM studies. The PM defines the cell's

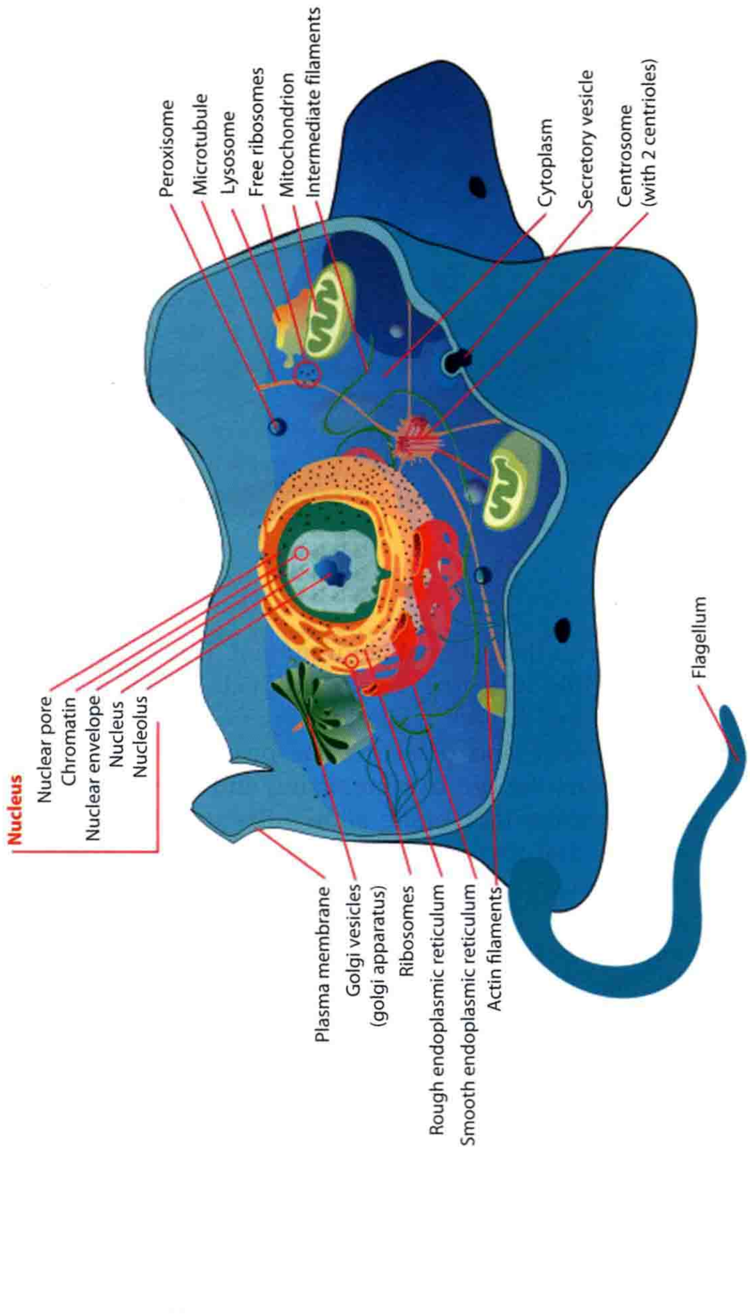

FIGURE 1.2 Cartoon depiction of the major components of an animal cell [2].

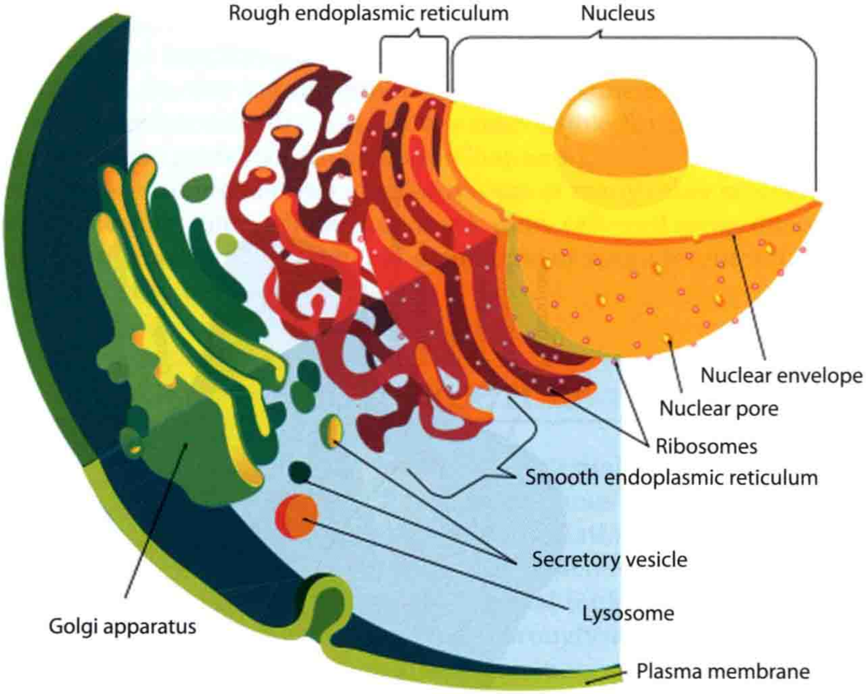

FIGURE 1.3 The endomembrane system of a 'typical' animal cell. The various membranes and compartments are connected to one another structurally, chemically, functionally, and developmentally [9].

boundary and its interaction with the external environment. It is responsible for transporting nutrients into the cell while allowing waste products to leave. Thus, it prevents unwanted materials from entering the cell while keeping needed materials from escaping. It maintains the pH of the cytosol, and preserves the proper cytosolic osmotic pressure. Proteins on the PM surface assist the cell in recognizing and interacting with neighboring cells. Other proteins on the plasma membrane allow attachment to the cytoskeleton and extracellular matrix, functions that maintain cell shape and fix the location of membrane proteins. The PM contains several characteristic functions and structures that can be used to identify PM fractions (Chapter 12). Tight junctions seal contacts between cells while desmosomes are adhesion sites between adjacent cells. Gap junctions (Chapter 14) contain hexagonal arrays of pores that allow communication between adjacent cells. Caveolae and coated pits (Chapter 14) are similar shaped PM invaginations that are involved in cell signaling and solute uptake, respectively. Plasma membranes are indeed complex entities.

Nuclear Envelope (Membrane)

The nuclear envelope is a double membrane surrounding a perinuclear space [12]. This space is probably contiguous with the lumen of the endoplasmic reticulum. The envelope has large nuclear pores (about 600 Å) that allow passage of large RNA-protein complexes out of the nucleus into the cytoplasm and movement of regulatory proteins from the cytoplasm into the nucleus.

Endoplasmic Reticulum (ER)

The endoplasmic reticulum (ER) is a complex network of cisternae or tube-like structures occupying a considerable percentage of the cell's internal volume [13]. The portion of the ER with attached ribosomes is known as the rough ER. It is the site for biosynthesis of non-cytoplasmic proteins that are either secreted, internalized into the lysosome, or become PM proteins. The portion of ER devoid of ribosomes is known as the smooth ER. Functions of the smooth ER include sterol biosynthesis, drug detoxification, calcium regulation, and fatty acid desaturation. A specialized ER, the sarcoplasmic reticula has but one function – regulation of intra-cellular calcium levels. The endoplasmic reticula were first seen by Keith R. Porter, Albert Claude, and Ernest F. Fullam in 1945 [14].

Golgi Apparatus

The Golgi apparatus [15,16] is a series of stacked, disk-shaped tubules. The Golgi is named after the man who discovered it in 1898, Carmillo Golgi. For this, in 1906 Golgi was awarded one of the first Nobel Prizes. It is in the Golgi that post-translational modification of glycoproteins synthesized originally in the ER and destined for secretion takes place. Other possible final destinations of proteins from the Golgi include incorporation into the PM or to the lysosome. Characteristic Golgi-resident enzymes involve sugar modification of proteins and include glucosidases and glycosyl transferases.

Lysosome

The lysosome contains some 40 hydrolytic enzymes whose function is to degrade macromolecules into component parts for re-use in the cell [17]. As a result lysosomes are often referred to as the 'cell's garbage disposal'. The organelle was discovered in the early 1950s and, in 1955, it was named lysosome, by Belgian cytologist Christian de Duve, for its ability to lyse membranes.

Peroxisome

Peroxisomes contain a battery of oxidative enzymes that are involved in breakdown of small molecules [18]. Important peroxisomal enzymes include D-amino acid oxidase and catalase, the enzyme responsible for the degradation of dangerous peroxides. Peroxisomes are also involved in drug detoxification and the biosynthesis of essential ether-phospholipids known as plasmalogens (discussed in Chapter 5). Peroxisomes were also discovered by Christian de Duve in 1967 [19]. For discovering lysosomes and peroxisomes, de Duve was awarded the 1974 Nobel Prize for Medicine (Figure 1.4).

Mitochondria

Mitochondria are the 'powerhouse' of the cell, producing most cellular ATP. The processes of electron transport and oxidative phosphorylation are housed in the highly folded mitochondrial inner (Cristae) membrane [20]. The mitochondrial aqueous interior chamber, called

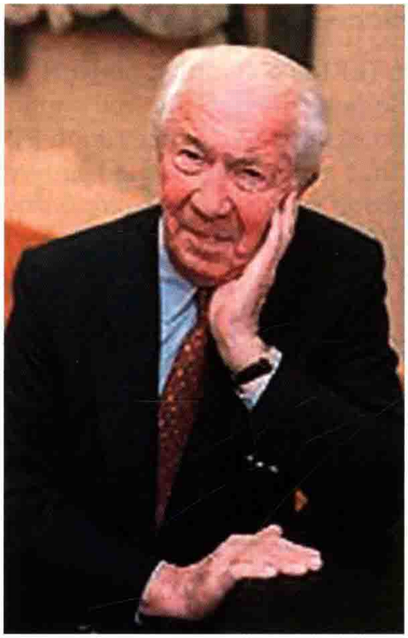

FIGURE 1.4 Christian de Duve (1917–). *Courtesy of Christian de Duve Institute of Cellular Pathology [24].*

the matrix, houses most of the enzymes involved in the Krebs Cycle (terminal steps in sugar oxidation) and β-oxidation (fatty acid oxidation) [21]. The inner mitochondrial membrane is surrounded by a second membrane, the outer mitochondrial membrane, which is very different and far less dynamic than the inner membrane. Mitochondria have been defined as 'semi-autonomous, self-replicating organelles', meaning they grow and replicate independently of the cell in which they are housed. This is a vestige left over from their origin as freely living prokaryotes that took up refuge inside larger prokaryotes about 1.5 billion years ago. This concept, known as Endosymbiont Theory [22,23], was originally ridiculed, but is now generally accepted. Mitochondria contain their own, albeit small, genome and code for a handful of mitochondrial membrane-protein components.

D. SIZE OF DOMAINS

Since membrane studies span a wide range of size and time domains, a variety of often esoteric instrumentation must be employed. The studies addressed in this book will range in size from Angstroms (Å) to microns (μm): Å (10^{-10} m), nm (10^{-9} m) and μm (10^{-6} m).

We will first address the question of size by asking whether someone with excellent vision can actually see a membrane. A person with excellent vision can resolve two spots about 0.1 mm apart.

$$0.1\ \text{mm} = 10^{-4}\text{m} = 10^{2}\ \mu\text{m} = 10^{5}\ \text{nm} = 10^{6}\ \text{Å}$$