Chapter 15
Characterization of Cholesterol Crystalline Domains in Model and Biological Membranes Using X-Ray Diffraction

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Introduction

Cholesterol is an important component of most biological membranes where it regulates structural and dynamic properties of the lipid bilayer through its direct interactions with membrane phospholipids (Chen et al. 1995; Leonard and Dufourc 1991; McIntosh 1978; Yeagle 1985). Free or unesterified cholesterol is amphipathic in nature and consists of a planar, alkyl-substituted, tetracyclic steroid nucleus, modified at carbon three by a polar hydroxyl substituent in the β-position. The polar hydroxyl group anchors cholesterol at the membrane surface, causing the molecule to orient in the membrane with its long-axis parallel to the surrounding phospholipid acyl chains (Yeagle 1985; Schroeder and Wood 1995). This orientation increases order in the upper acyl chain region of the membrane while decreasing packing constraints among the terminal methyl segments located in the hydrocarbon core, effectively *condensing* the spatial arrangement of phospholipids within the membrane bilayer (Yeagle 1985; Schroeder and Wood 1995; Shinitzky and Inbar 1976). The effects of cholesterol on the conformation and rotational dynamics of neighboring molecules are highly dependent on the acyl chain composition and structural integrity of membrane phospholipids (Tulenko et al. 1998). Oxidative modification of membrane lipid acyl chains, for example, can affect the behavior of cholesterol and even its tendency to associate with other sterol molecules.

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Membrane Effects of Cholesterol Enrichment

The amount of cholesterol present in a biological membrane influences its biophysical properties, including the activity of membrane-restricted proteins. Changes in membrane cholesterol content can alter the conformation and activity of various channel proteins, including calcium channels (Bialecki and Tulenko 1989) and potassium channels (Bolotina et al. 1991). Cholesterol enrichment of the cell membrane has also been shown to inhibit Na^+/K^+ ATPase activity in erythrocytes (Broderick et al. 1989), endothelial cells (Lau 1994), and renal cells (Yeagle et al. 1988). In vascular smooth muscle cells derived from an animal model of dietary atherosclerosis, calcium transport mechanisms and basal intracellular calcium levels were observed to change as a function of increased membrane cholesterol content (Gleason et al. 1991). In addition, cholesterol enrichment has been shown to alter the conformation of calcium-activated potassium channels, forcing the ion channel pore to favor the closed state under otherwise normal stimulatory conditions (Chang et al. 1995). These functional effects of cholesterol enrichment correlated directly with changes in structural stress and lateral elastic stress energy (Chang et al. 1995). Changes in cholesterol content have also been shown to influence G-protein coupled receptors, including the serotonin receptor (Shrivastava et al. 2010). Collectively, these observations provide compelling evidence for the hypothesis that membrane cholesterol levels must be maintained within certain physiologic limits in order to ensure proper cell and cell membrane function.

Lipid Rafts

The cell plasma membrane is a complex structure consisting of numerous microdomains assembled from specific lipid and protein constituents. These membrane domains compartmentalize cellular processes by serving as organizing centers for the assembly of signaling molecules while also modulating membrane fluid dynamics and regulating protein trafficking, receptor function, and other cellular activity such as neurotransmission.

One type of domain that has been the subject of intensive investigation is the *lipid raft*, which is more highly-ordered as compared to the surrounding membrane bilayer (Simons and Toomre 2000). Lipid rafts contain 3–5 times the amount of cholesterol as compared to the surrounding bilayer and are also enriched in sphingolipids, particularly sphingomyelin, which interacts favorably with cholesterol due to its accommodating headgroup structure and the highly-saturated nature of its hydrocarbon chains. Although not all phospholipids associated with lipid rafts are fully saturated, the acyl chains present in this domain are typically more saturated and more tightly-packed than those in the surrounding membrane bilayer. Cholesterol, by virtue of its inherent structural properties as well as its affinity for lipids with more rigid acyl chains, plays an essential role in stabilizing lipid rafts.

Lipid rafts possess properties consistent with the gel state, including extended acyl chains and a relatively high melting temperature, but also properties associated with the liquid crystalline state, such as rapid lateral molecular mobility (Brown and London 2000; Ostermeyer et al. 1999). These membrane domains also host specific cellular proteins and mediate a variety of biologic processes, including signal transduction, adhesion, and sorting of membrane components. The insulin receptor, for example, is known to form functional dimers in lipid rafts but not in other regions of the membrane. T cell antigen receptor activation on the surface of T lymphocytes is regulated by their association with lipids rafts. Viruses, as obligate intracellular parasites, bind to cellular receptors expressed in lipid rafts in order to gain access to target cells. Many vertebrate cell types also contain specialized lipid rafts known as caveolae, which appear (by microscopic analysis) as small, flask-shaped invaginations of the plasma membrane. These rafts are enriched with cholesterol, sphingomyelin, and unique proteins, such as caveolin, and engage in various cell functions, including endocytosis and signal transduction (Edidin 1997).

Cholesterol Domains in Model and Biological Membranes

The systematic addition of cholesterol to biological membranes eventually results in lateral phase separation and the formation of membrane-restricted cholesterol domains (Tulenko et al. 1998; Bach et al. 1998; Engelman and Rothman 1972; Houslay and Stanley 1982; Rice and McConnell 1989; Ruocco and Shipley 1984; Slotte 1995a, b). In model membranes prepared largely from lecithin, cholesterol was shown to aggregate into clusters at cholesterol-to-phospholipid mole ratios greater than 0.3:1 (Engelman and Rothman 1972) and to form separate domains at ratios greater than 1:1 (Houslay and Stanley 1982). Similar effects have been observed in well-defined lipid monolayer systems using various microscopy approaches (Rice and McConnell 1989; Slotte 1995a, b). Cholesterol domains have also been characterized in membrane bilayers using small angle X-ray diffraction and other biophysical techniques. Ruocco and Shipley showed that increasing the cholesterol content of model membrane bilayers to levels greater than 50 mol % resulted in the formation of an immiscible cholesterol monohydrate phase, with a characteristic unit cell periodicity of 34 Å, that was coexistent with a bulk, liquid-crystalline lipid bilayer phase (Ruocco and Shipley 1984). The repeat unit associated with the cholesterol phase corresponds to a tail-to-tail arrangement of cholesterol molecules, as the long axis of cholesterol monohydrate is 17 Å in the crystalline state (Craven 1976). This interpretation has been confirmed in other model membrane systems, as well as select native membrane preparations such as myelin membranes, using a variety of techniques (Schroeder and Wood 1995; Bloom and Thewalt 1995; Harris et al. 1995; Hui 1995; Tocanne 1992; Kirschner and Caspar 1972).

Lipid rafts isolated from neuronal cell membranes, and identified as detergent-insoluble membrane fractions, were shown to contain relatively low amounts of

sphingomyelin but very high amounts of cholesterol (Maekawa et al. 1999). Epand and coworkers extended this work and showed that the formation of cholesterol-rich domains could be induced in model membranes by introducing a neuronal protein—namely NAP-22, a myristoylated, calcium-dependent , calmodulin-binding protein found largely in the synapse and shown to be a major component of neuron-associated, detergent-insoluble, low-density membrane fractions. Differential scanning calorimetry analysis demonstrated that NAP-22 changed the shape and enthalpy of the phase transition of phosphatidylcholine and induced the appearance of cholesterol "crystalline" domains in membranes composed of phosphatidylcholine with either saturated or unsaturated acyl chains. Using atomic force microscopy, NAP-22 was further shown to cause a marked change in the surface morphology of dioleoylphosphatidylcholine bilayers containing cholesterol at 40 mol %. In the absence of protein, the membrane bilayer appeared as a smooth structure of uniform thickness; the addition of NAP-22 resulted in the formation of a more convoluted surface consisting of raised bilayer domain structures measuring approximately 1.5 nm in height (Epand et al. 2001).

Role of Cholesterol Domains in Membrane Function

Cholesterol is typically associated with separate kinetic domains (or pools) and is thus considered to be distributed non-randomly within the plasma membrane (Yeagle 1985; Liscum and Underwood 1995; Phillips et al. 1997; Schroeder et al. 1991, 1995). Regulation of the size and physico-chemical properties of these kinetic domains may influence extracellular and intracellular cholesterol transport pathways (Schroeder et al. 1991; Bretscher and Munro 1993). Investigators have proposed that cholesterol domains may modulate the activity of membrane proteins that localize specifically to cholesterol-rich domains (*e.g.*, nicotinic acetylcholine receptor, human erythrocyte band 3 protein, glycophorin, as well as Na^+/K^+-ATPase) or cholesterol-poor domains (*e.g.*, Ca^{2+}ATPase) (see Mukherjee and Chattopadhyay for review (Mukherjee and Chattopadhyay 1996)). Sterol-rich regions have also been hypothesized to play a crucial role in other cellular functions, including signal transduction, cell adhesion, cell motility, and the sorting and trafficking of membrane components (Janes et al. 2000; Langlet et al. 2000; Simons and Ikonen 1997, 2000).

Membrane Structural Analysis Using Small Angle X-ray Diffraction

The use of X-ray diffraction approaches to study the structural properties of biological membranes has been well established. Membrane diffraction studies were first reported in the 1930s; however, this area of inquiry remained somewhat esoteric

until the 1960s, when the field experienced rapid growth (Franks and Levine 1981). Since that time, small angle X-ray diffraction has been used extensively to study various model and native membrane preparations.

In order to appreciate the use of X-ray diffraction approaches in analyzing membrane structure, it is important to consider the *lipid bilayer theory*. According to this theory, lipids that comprise a membrane are arranged in a bilayer structure as a result of their amphipathic properties. All typical lipids have a polar, hydrophilic headgroup region and a nonpolar, hydrophobic fatty acyl chain region. In order to avoid energetically unfavorable interactions with water, lipids will associate with one another such that their headgroups form two surfaces in contact with the surrounding aqueous environment, with their acyl chains oriented into the space between the two surfaces. The acyl chain region of a bilayer formed in this manner is called the membrane or hydrocarbon core, while the hydrophilic surfaces are known as the membrane headgroup layers (Blaurock 1982). If cholesterol is present (which is true of almost all naturally occurring membranes), this molecule is positioned almost entirely within the acyl chain region of the bilayer.

This specific arrangement of membrane lipids is important in that it serves as the basis for the structural continuity of a membrane repeat unit. If membranes are "stacked" into multiple layers, this basic bilayer structure becomes a periodic function that yields coherent scattering in diffraction analyses. The unit cell of such a system is represented by the membrane lipid bilayer that is repeated in these preparations. Numerous X-ray diffraction experiments have been conducted using membrane multibilayers, including myelin membranes (Blaurock 1971; Moody 1963), disk membranes from the outer segments of retinal rod cells (Blaurock and Wilkins 1972; Corless 1972), erythrocyte ghosts (Knutton et al. 1970), and artificial multilayers derived from the sarcoplasmic reticulum (Dupont and Hasselbach 1973; Worthington and Liu 1973). Some membrane systems, such as nerve myelin membranes and rod outer segment membranes, occur naturally as repeating, multibilayer structures. Fiber cells of the ocular lens, as discussed below, also appear to be organized into regular, repeating membrane units, making them particularly well-suited to X-ray diffraction analysis.

X-ray diffraction analysis of a multibilayer membrane sample results in the production of discrete diffraction peaks also known as Bragg reflections. These reflections result from the coherent (constructive) scattering of secondary X-rays produced by atoms comprising a sample. Coherent scattering from membranes follows the same rules as required for the diffraction of crystals: (1) the spacing between the scattering planes must be roughly equal to the wavelength of the incident X-rays, (2) the scattering centers (membrane layers) must be spatially distributed in a highly regular manner, and (3) the repeating membrane units must be oriented so that the diffraction angle (θ) satisfies Bragg's law, $h\lambda = 2d\ sin\theta$, where h is the diffraction order, λ is the wavelength of the X-ray radiation, d is the membrane lipid bilayer unit cell periodicity, and θ is the Bragg angle equal to one-half the angle between the incident beam and scattered beam. The relationship of Bragg's law to the diffraction analysis of a membrane multibilayer sample is illustrated in Fig. 15.1. In this case, the individual

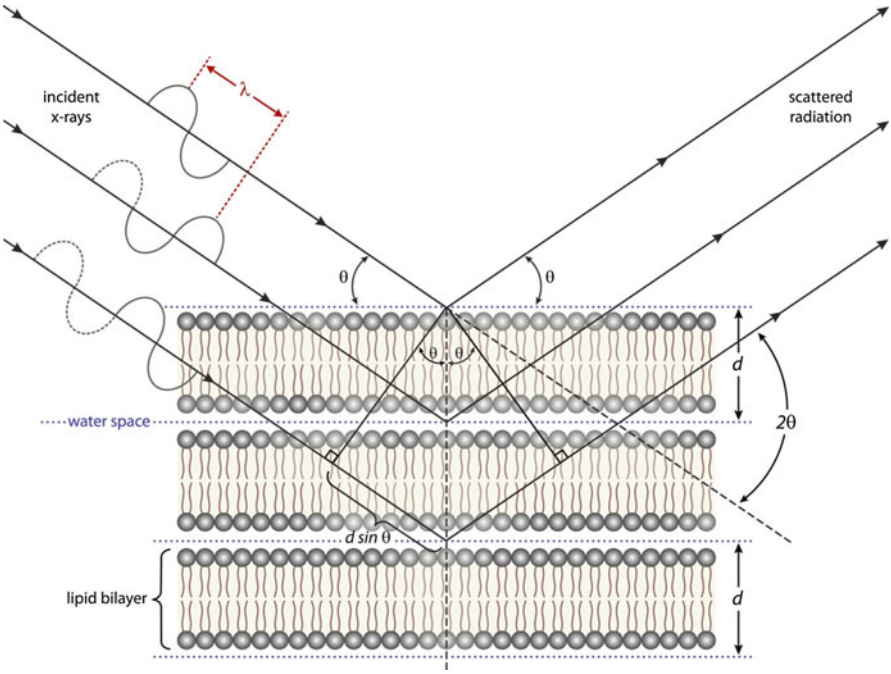

Fig. 15.1 Schematic representation of membrane bilayers as an X-ray diffraction lattice. The unit cell periodicity, *d*, represents the distance spanning a single bilayer plus half the water space on each side of the bilayer. θ is the Bragg angle and is equal to one-half the angle between incident and scattered radiation

lipid bilayer represents the minimum volume of information that is being repeated in the sample (*i.e.*, unit cell periodicity). The unit cell periodicity, *d*, is often referred to as the *d*-space, and represents the distance from the center of one water space to the next across the lipid bilayer.

X-Ray Diffraction Method

A typical X-ray diffraction experiment consists of placing a multilammelar membrane specimen into a monochromatic, collimated beam of X-rays and measuring the intensity of the scattered radiation (Fig. 15.2). Diffraction occurs only when the plane of each sample bilayer is oriented around an axis perpendicular to the incident X-ray beam. After orienting a membrane sample using relatively gentle centrifugation approaches (Franks and Levine 1981), the membrane sample is positioned relative to the incident X-ray beam to allow for the parallel alignment of the repeating membrane planes with the imaginary Bragg planes, thus achieving the specific angles required for diffraction as described by Bragg's law.

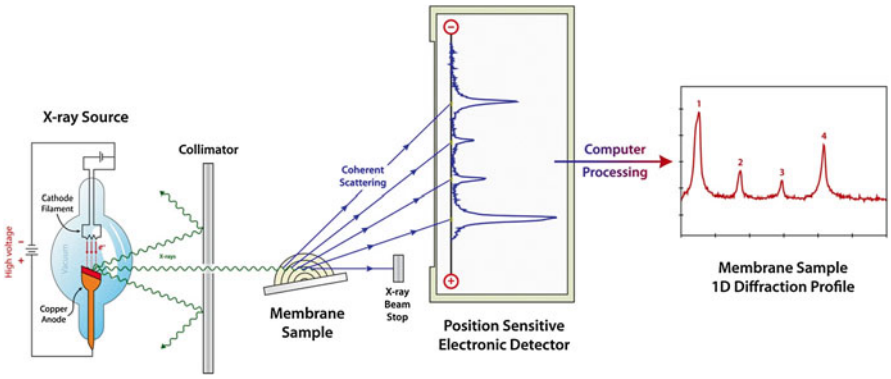

Fig. 15.2 Schematic representation of the small angle X-ray scattering method. Monochromatic radiation ($\lambda = 1.54$ Å) is produced by a high-brilliance, microfocus generator. An oriented sample is placed on a curved mount at near-grazing incidence with respect to the focused beam. Coherent scattering data are then collected on a one-dimensional, position-sensitive electronic detector

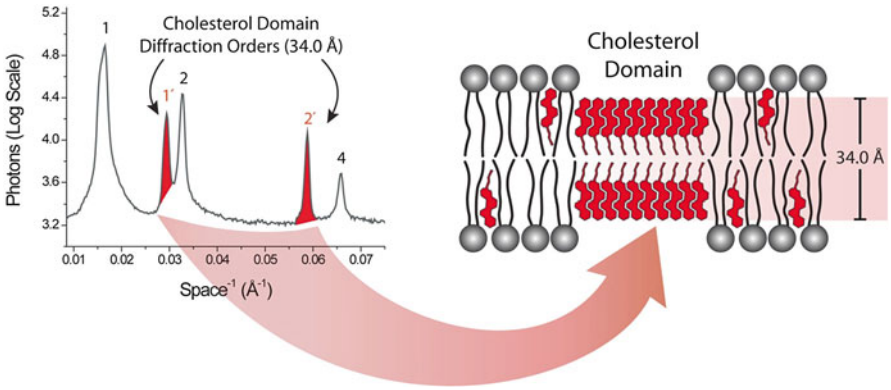

Fig. 15.3 Identification of membrane cholesterol domains using small angle X-ray diffraction approaches. Cholesterol domains yield characteristic diffraction peaks (1′ and 2′) that correspond to a unit cell periodicity (*d*-space) of 34 Å

In our laboratory, membrane diffraction is accomplished by aligning the sample at grazing incidence with respect to a collimated, monochromatic X-ray beam produced by a Rigaku Rotaflex RU-200, high-brilliance microfocus generator (Rigaku Americas, The Woodlands, TX). The fixed geometry beamline utilizes a single Franks mirror providing nickel-filtered radiation ($K\alpha_1$ and $K\alpha_2$ unresolved) at the detection plane. Diffraction data are collected on a one-dimensional, position-sensitive electronic detector (Hecus X-ray Systems, Graz, Austria) at a sample-to-detector distance of 150 mm and calibrated using cholesterol monohydrate crystals.

The presence of cholesterol domains in a given membrane sample results in the production of a distinct set of Bragg peaks having a singular unit cell periodicity of 34 Å (Fig. 15.3). Under typical temperature and relative humidity conditions,

the second-order cholesterol domain peak is well-delineated from other, neighboring cholesterol or phospholipid diffraction peaks and can be used to quantitate relative cholesterol domain peak intensity (calculated as the fraction of total peak area).

Cholesterol Domains in Vascular Smooth Muscle Cell Membranes

Atherosclerosis is the product of endothelial dysfunction, inflammation, and excessive lipid accumulation in the arterial wall (Libby 2002). Given its principal role in the structural and functional properties of low-density lipoproteins (LDL), cholesterol has been the primary focus of much of the research conducted in this field. Cholesterol exists in free form, as previously described, or as cholesteryl esters, which are formed by the action of acyl-coenzyme A (CoA)-cholesterol acyl transferase (ACAT). This enzyme catalyzes the covalent attachment of a fatty acid moiety to the hydroxyl group on cholesterol, converting the molecule into a more hydrophobic form for improved storage and transport.

Cholesterol uptake into cells is regulated by the expression of LDL receptors. Through various feedback mechanisms, LDL expression is reduced when cholesterol biosynthesis occurs at adequate levels in the cell. If ACAT is inhibited or rendered ineffective by some perturbation, free cholesterol levels become elevated in the cell. Under such conditions, cholesterol also accumulates in the plasma membrane where it can aggregate into discrete, crystalline domains. These domains are believed to precede the development of microscopic cholesterol crystals that are typically observed in the extracellular space associated with the atherosclerotic plaque (Kellner-Weibel et al. 1999; Small 1988). These cholesterol crystals are toxic and contribute to the instability of the atherosclerotic lesion by increasing local inflammation and plaque mass (Small 1988).

Using X-ray diffraction approaches, we directly examined the effects of cholesterol enrichment on membrane lipid structural organization in cultured smooth muscle cells and cells obtained *ex vivo* from an animal model of dietary atherosclerosis (Tulenko et al. 1998). The comparative effects of cholesterol enrichment in these separate systems were remarkably consistent. Following 9 weeks of feeding with a cholesterol-enriched diet, the cholesterol-to-phospholipid mole ratio measured in aortic smooth muscle cell membranes increased from 0.4:1 to approximately 1:1. Under such atherosclerotic-like conditions, prominent cholesterol domains (identified by their characteristic periodicity of 34 Å) could be observed in the smooth muscle cell plasma membranes (Tulenko et al. 1998). The formation of cholesterol domains was also reproduced in this study using membranes reconstituted as binary mixtures of bovine cardiac phosphatidylcholine and cholesterol at a cholesterol-to-phospholipid mole ratio of 1:1 (Tulenko et al. 1998).

In another study, cultured mouse peritoneal macrophage foam cells were treated with an ACAT inhibitor, which induced the formation of free cholesterol crystals that extended away the cell membrane with various morphologies, including plates,

needles and helices (Kellner-Weibel et al. 1999). With the use of X-ray diffraction approaches, the early stages of crystal formation could be identified in whole cell and isolated membranes obtained from either diseased tissue *ex vivo* or cultured cells *in vitro* following ACAT inhibition (Tulenko et al. 1998; Kellner-Weibel et al. 1999). Preventing crystal formation is an important goal as cholesterol in this state is pro-inflammatory and does not respond well to pharmacologic interventions that promote lesion regression due to its high stability (Small 1988; Katz et al. 1982).

Cholesterol Domains in Model Membranes Exposed to Atherogenic Conditions

The formation of cholesterol crystalline domains in the membrane can also occur in the absence of sterol enrichment. In particular, such domains can form following oxidative modification to membrane lipids in a manner that can be inhibited with certain lipophilic antioxidants or stimulated with pro-oxidant agents (Jacob and Mason 2005; Mason et al. 2006; Jacob et al. 2013). A similar increase in cholesterol domains with oxidative stress was also observed under conditions of hyperglycemia (Self-Medlin et al. 2009). In these models of disease, the formation of well-defined cholesterol domains did not require a change in the overall membrane cholesterol content but were directly related to levels of lipid hydroperoxides generated during oxidative stress. These findings indicate that the interactions of cholesterol with surrounding phospholipids are influenced by their physico-chemical properties, including chemical modifications resulting from their interactions with reactive oxygen species. We also observed that changes in membrane width and even cholesterol domain formation are highly dependent on the length and degree of phospholipid acyl chain composition (Tulenko et al. 1998). Finally, cholesterol itself can undergo oxidative modification during various disease processes. At high levels, these oxidized sterols also form domains and extracellular crystals with dimensions that differ from that of non-oxidized forms but that still cause apoptosis (Phillips et al. 2001; Geng et al. 2003).

Cholesterol Domains in Ocular Lens Fiber Cell Membranes

The human ocular lens has been a particularly interesting tissue for analysis of cholesterol domains in the plasma membrane. Unlike cells associated with atherosclerosis, it appears that the presence of cholesterol crystalline domains is essential for normal ocular function and light transparency. Through the controlled regulation of its shape, the ocular crystalline lens allows for light to be efficiently transmitted through the eye and focused onto the retina. The lens is an encapsulated structure consisting almost entirely of a large number of rigid, elongated cells known as lens fibers or fiber cells, which are produced by the differentiation of a single layer of

epithelial cells located just beneath the anterior surface of the lens capsule. These cells are deposited in successive layers through a process that begins in early embryogenesis and continues throughout life. Existing fiber cells are displaced toward the center of the lens as new layers are formed. In the *lens nucleus*, mature fiber cells are compacted into the center of the lens; cells peripheral to this region, including new and mitotically active cells of the adult lens, are collectively referred to as the *lens cortex*. Fibers cells eventually lose all subcellular organelles during their progressive displacement toward the lens nucleus. As a result, the plasma membrane becomes the only substantive organelle of the adult lens (Rafferty 1985). A unique biochemical characteristic of the fiber cell plasma membrane is its relatively high level of free cholesterol. The cholesterol-to-phospholipid mole ratio of the fiber cell membrane ranges from 1 to 2 in the cortex to as high as 3–4 in the lens nucleus (Li et al. 1985, 1987). This stands in sharp contrast to the levels of cholesterol found in other mammalian plasma membranes, which range between 0.5 and 1.0. The fiber cell plasma membrane is also distinct from other biologic membranes in that it contains only trace amounts of polyunsaturated fatty acid (Broekhuyse and Soeting 1976) and, in the human lens, a phospholipid composition of more than 50 % sphingomyelin and sphingomyelin derivatives (Byrdwell and Borchman 1997; Byrdwell et al. 1994).

The unusual lipid composition of fiber cell plasma membrane makes it an intriguing biologic system for conducting structural studies. Moreover, fiber cells can be efficiently removed from the lens and plasma membranes isolated for X-ray diffraction analysis. It was predicted, based on previous studies in model membrane systems, that these biologic membranes would be organized as a "mosaic of phospholipid and cholesterol patches" (Li et al. 1985).

Using small angle X-ray diffraction approaches, we observed that cholesterol domains are clearly present in both reconstituted and intact (protein-containing) fiber cell plasma membrane preparations (Fig. 15.4). These domains were identified by their characteristic, 34 Å repeat orders, which remained stable over a broad range of temperature and relative humidity conditions (Jacob et al. 1999). By contrast, the dimensions of the surrounding liquid crystalline phase increased by as much as 30.9 Å (60 %) in reconstituted lens plasma membrane. Interestingly, the dimension of the sterol-poor region of the membrane was less affected by experimental conditions in the *intact* fiber cell plasma membranes. Thus, while the presence of protein is not necessary for the formation of immiscible cholesterol domains, it does appear to significantly influence both the size of the cholesterol domains and the dimensions of the surrounding sterol-poor region. In these membrane preparations, the ratio of cholesterol to phospholipid exceeded 2:1 under normal conditions.

The functional role for discrete cholesterol regions in ocular lens fiber cell plasma membrane is an intriguing question. The essential activity of the lens fiber cell is to facilitate the efficient transmission of visible light through the eye. By ordering membrane lipid constituents, higher cholesterol levels may provide such transparency. Infrared spectroscopy approaches have demonstrated that the highest membrane cholesterol content is associated with the center or more visually-significant region of the ocular lens (Borchman et al. 1996). Another role for cholesterol domains may be to interfere with the membrane association of the protein

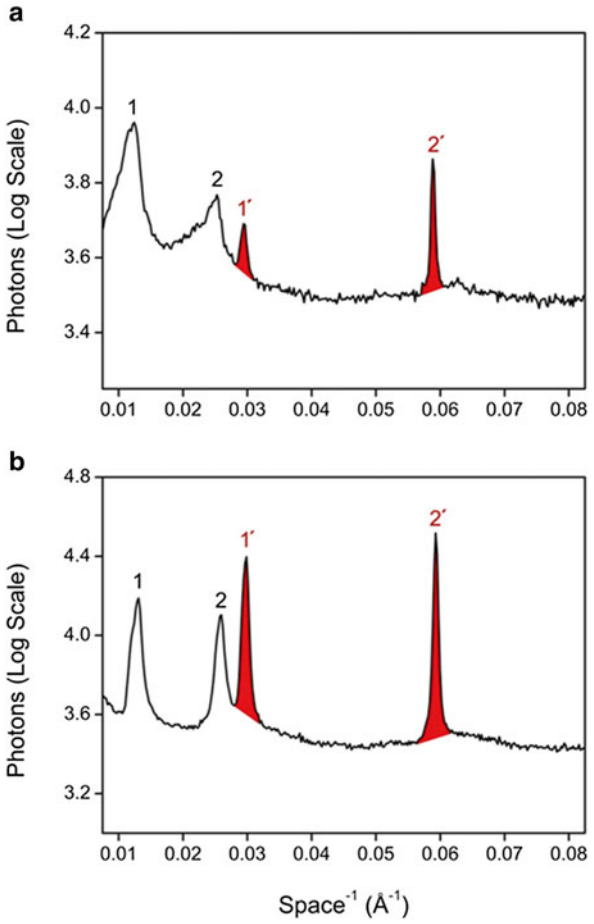

Fig. 15.4 Representative X-ray diffraction patterns obtained from intact (A) and reconstituted (B) human ocular lens fiber cell plasma membranes. Data were collected on a one-dimensional, position-sensitive electronic detector at 20 °C, 92 % RH. Diffraction peaks labeled as 1′ and 2′ correspond to immiscible cholesterol domains; other peaks correspond to the surrounding membrane lipid bilayer. This research was originally published in the *Journal of Biological Chemistry*. R.F. Jacob, R.J. Cenedella, and R.P. Mason. Evidence for distinct cholesterol domains in fiber cell membranes from cataractous human lenses. *J Biol Chem*. 2001; **276**:13573-13578. © the American Society for Biochemistry and Molecular Biology

crystallin (especially α-crystallin), an important feature of human and experimental animal cataracts (Chandrasekher and Cenedella 1995). In fact, cataractogenesis can be accelerated in an animal model by reducing membrane cholesterol content in the lens with specific biosynthesis inhibitors (Cenedella and Bierkamper 1979). The results of these membrane structure studies suggest that the coexistence of distinct sterol-rich and -poor regions may interfere with the ability of extrinsic proteins to aggregate at the membrane surface (Jacob et al. 1999).

Conclusion

Small-angle X-ray diffraction approaches have been used to successfully evaluate the organization of lipids in plasma membranes derived from distinct mammalian cell types, including arterial smooth muscle cells and ocular lens fiber cells.

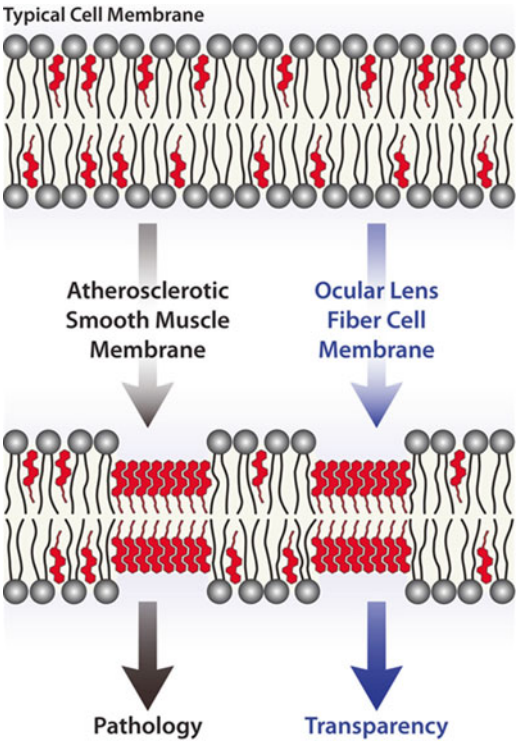

Fig. 15.5 A schematic model describing the formation of immiscible cholesterol domains in atherosclerotic-like smooth muscle cell plasma membranes following cholesterol enrichment and leading to disease. The cholesterol microdomain has a unit cell periodicity of 34 Å and coexists in the same plane as the surrounding sterol-poor region. At the same time, these same cholesterol domains are essential for normal ocular lens function and transparency. These studies show cholesterol can self-associate into immiscible domains within the plasma membrane, a phenomenon that contributes to both physiologic and pathologic cellular processes

These studies show that at elevated cholesterol concentrations or under conditions of oxidative stress, cholesterol can self-associate into immiscible domains within the plasma membrane, a phenomenon that contributes to both physiologic and pathologic cellular processes (Fig. 15.5). In fiber cell plasma membranes isolated from the ocular lens, by contrast, cholesterol domains appear to be essential to normal physiology. The unique structural heterogeneity of the lens fiber cell plasma membrane appears to facilitate lens transparency while interfering with cataractogenic aggregation of soluble lens proteins at the membrane surface. Taken together, these analyses provide examples of the complex roles that sterol-rich domains may have in mammalian plasma membranes. Combined with the findings from various other laboratories, these data support a model of the membrane in which cholesterol aggregates into structurally distinct regions that regulate the function of the cell membrane and may contribute to mechanisms of disease.

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