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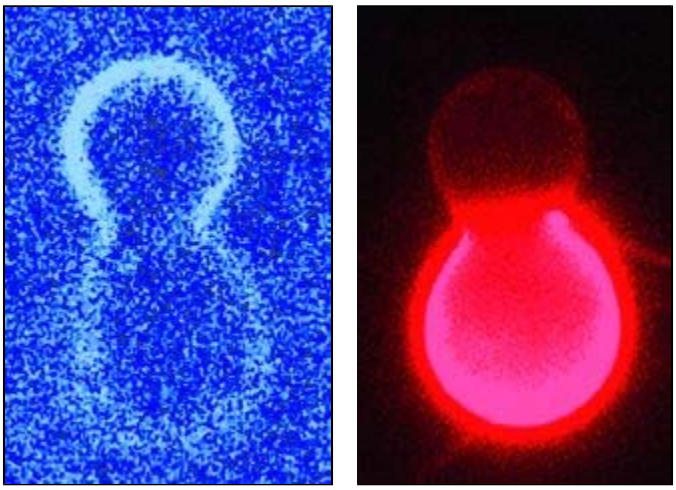
FROM THE ACS MEETING

CHEWING THE FAT ABOUT CHOLESTEROL

Molecule plays a key role in controlling the organization and fluidity of cell membranes



SPLITTING UP In this model membrane made of just three lipid components, a fluid and ordered cholesterol-rich domain (blue) separates from a fluid and disordered phospholipid-rich domain (red). Both images are of the same vesicle, but using different fluorescent reporters.

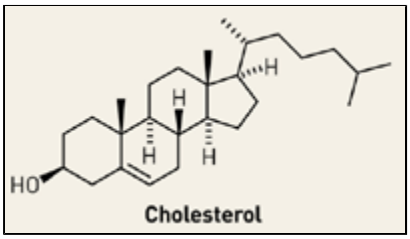
COURTESY OF GERALD FEIGENSON

AMANDA YARNELL, C&EN WASHINGTON

Despite being much maligned in the popular press, cholesterol plays a crucial role in organizing cell membranes and controlling their physical properties. At a symposium at last month's American Chemical Society national meeting in Anaheim, Calif., physical chemists, physicists, chemical engineers, and biologists connected to discuss how cholesterol tweaks membrane properties and how such adjustments

could be involved in diseases such as gallstone formation and atherosclerosis.

Cells count on membranes to offer protection from the outside world and to cordon off specialized internal compartments, forming organelles like mitochondria. Simplistic cartoons of cell membranes often show a bilayer of amphiphilic lipids, packed with their waxy tails together and their polar headgroups facing outward. But the membrane is actually a far more complicated place.



Cholesterol

In reality, cell membranes are crowded with proteins and contain a bewildering variety of lipids. Cholesterol is one of the most important of these lipids. In some cases accounting for up to half of the lipids in the membrane, this rigid sterol molecule intercalates among the tightly packed acyl chains of other lipids, acting as a sort of membrane plasticizer.

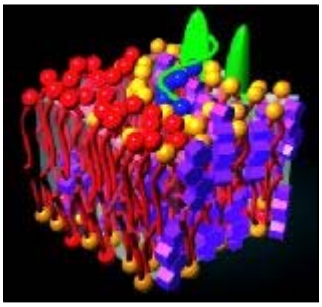
To make things more complicated, lipids aren't evenly scattered throughout the bilayer: Different membranes contain different catalogs of lipids, and within each membrane, certain lipids--particularly cholesterol and sphingomyelin--seem to stick together, forming domains called "rafts" that float amid a sea of more fluid lipids.

Biologists have suggested that these cholesterol-rich domains act as platforms for a range of crucial cellular processes, including signaling and vesicle trafficking. But because they can't be isolated in pure form, direct characterization of these domains has proven difficult. Instead, scientists have turned to model systems to study raft formation.

AT A DIVISION of Colloid & Surface Chemistry-sponsored symposium held in Anaheim, biophysicist Gerald W. Feigenson of Cornell University described his lab's efforts to investigate lipid behavior in large vesicles with membranes made up of a mixture of three lipids: dioleoylphosphatidylcholine, sphingomyelin, and cholesterol. Using a fluorescent

analog of cholesterol, they observe that the sphingomyelin and cholesterol in this model cell membrane do indeed tend to partition away from the dioleoylphosphatidylcholine, forming a cholesterol-rich region that pinches off from the rest of the vesicle.

In another example, biophysicist Stephen R. Wassall of Indiana University-Purdue University, Indianapolis, described the partnering preferences of a different class of lipids known as polyunsaturated fatty acids. Wassall has used solid-state nuclear magnetic resonance spectroscopy and X-ray diffraction to show that introduction of polyunsaturated lipids reduces the solubility of cholesterol in membranes. In fact, in membranes that contain both polyunsaturated fatty acids and cholesterol, Wassall reported that lipids tend to separate into two phases: one rich in cholesterol but poor in polyunsaturated fatty acids, and the other poor in cholesterol but rich in polyunsaturated fatty acids.



AFLOAT In this conceptual cartoon of a lipid "raft," cholesterol (purple), proteins (green), and lipids such as sphingomyelin cluster within a sea of other lipids.
COURTESY OF DANIEL HARRIES

"This implies that polyunsaturated phospholipids separate from cholesterol, thereby promoting raft formation," Wassall remarked. Polyunsaturated fatty acids' ability to induce raft formation may be associated with the myriad of health benefits that have been attributed to diets high in these lipids, he suggested.

Lipids do tend to cluster into domains in these model systems. But these and other simple models don't take membrane proteins into account. In fact, scientists are divided over what symposium co-organizer and physical chemist Daniel Harries of the National Institute of Child Health & Human Development's (NICHD) Laboratory of Physical & Structural Biology calls the "chicken and egg" question of raft assembly: Do lipids alone form domains that

then attract proteins, or do proteins form rafts around themselves by attracting specific lipids?

Physical chemist Avinoam Ben-Shaul of Hebrew University of Jerusalem has used computer simulations to demonstrate that a protein can, in fact, trigger local variations in lipid composition. In these simulations, the protein attracts some lipids while repelling others, Ben-Shaul reported. Such preferences can result in complete separation of lipid phases, particularly if the lipids have inherent preferences for specific lipid neighbors, as cholesterol does, he added.

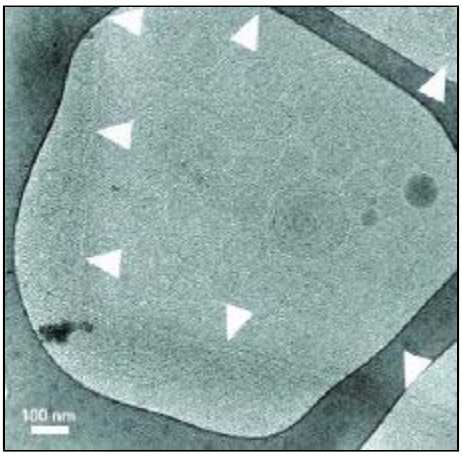
Besides playing a role in raft formation, cholesterol affects membranes' physical properties. Physicist Horia I. Petrache of the Laboratory of Physical & Structural Biology at NICHD presented data demonstrating that subtle changes in the cholesterol molecule can have dramatic effects on membrane elasticity. On the basis of X-ray diffraction and osmotic stress experiments, Petrache showed that membranes containing cholesterol are more resistant to bending than those containing sterols whose structures differ only slightly from cholesterol. His collaborators at the University of Arizona have used NMR to demonstrate the same effect. "We think this has implications in genetic diseases that impair cholesterol production from its precursors," Petrache said.

On the other hand, cholesterol-rich domains have been implicated in a staggering number of diseases. Biologist Joshua Zimmerberg of the Laboratory of Cellular & Molecular Biophysics at NICHD reported that cholesterol-rich membrane domains are required for the entry of the influenza virus into its host.

In addition, "cholesterol-rich domains--maybe even cholesterol-pure ones--are likely the precursors to gallstones," which are largely made up of crystalline cholesterol, argued chemical engineer Steven P. Wrenn of Drexel University, who co-organized the symposium with Harries and chemical engineer Dganit Danino of Technion--Israel Institute of Technology, in Haifa. Although Wrenn admitted to being skeptical of

the idea at first, he said his own experiments have led him to conclude that "such cholesterol crystals seem to be originating in the membrane itself."

CHOLESTEROL CAN be obtained from one's diet or synthesized in the body. Regardless of the source, cholesterol is packaged in lipoprotein and delivered to cell membranes throughout the body via the bloodstream. Excess cholesterol is carried in the membrane of phospholipid vesicles and secreted into the gall bladder. But if cholesterol is allowed to phase-separate in the membranes of these vesicles, it can nucleate and develop into cholesterol crystals that eventually grow into painful gallstones.



FIRST GLIMPSE In this transmission electron micrograph of human bile, vesicles with cholesterol-rich membranes (concentric circles) cluster around a nascent cholesterol crystal (perimeter marked by white arrowheads) that will soon become a gallstone.
COURTESY OF YESHAYAHU TALMON

Wrenn and gastroenterologist Sum P. Lee of the University of Washington, Seattle, have developed a method that detects the earliest events of cholesterol nucleation using fluorescence resonance energy transfer (FRET). A complementary technique that uses cryogenic transmission electron microscopy to monitor cholesterol crystallization in human bile has been developed by chemical engineer Yeshayahu Talmon of Technion, gastroenterologist Fred M. Konikoff of Israel's Tel Aviv University, and Danino. "We're using this approach to investigate the factors that influence cholesterol nucleation and crystal formation," Talmon said. He noted that his team has already used the method to image growing cholesterol monohydrate crystals in human bile.

A similar mechanism for cholesterol nucleation and crystallization may be at work in the formation of atherosclerotic plaques, noted vascular biologist Thomas N. Tulenko of Thomas Jefferson University College of Medicine, in Philadelphia. Such plaques--the formation of which can lead to dangerous narrowing of the arteries--are studded with tiny cholesterol crystals. Tulenko presented data indicating that the plasma cell membranes of certain types of heart cells in rabbits fed high-cholesterol diets are enriched in cholesterol. On the basis of X-ray diffraction and FRET assays, Tulenko concluded that cholesterol thickens the membrane and induces the formation of immiscible cholesterol domains that initiate the nucleation of cholesterol crystals found in plaques.

A better understanding of the chemistry of cholesterol in the body may one day lead to insights into controlling or preventing plaque buildup and gallstone formation, Wrenn said.

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