Of Craters and Crevices

A scientist challenges traditional views of cell shapes

By DAMARIS CHRISTENSEN

Suppose a team of cell biologists could shrink themselves and walk upon the surface of a cell. Like travelers on the skin of our planet, these explorers would expect to see a generally smooth surface, studded here and there with proteins heaped into mountain ranges and buttes.

But the biologists might not be walking on a sphere, theorizes Tomas Landh, a biomaterials professor at the State University of New York at Buffalo. Instead, they might find deep, symmetrical labyrinths that fold away into the depths of the cell.

"They're probably going to get lost," warns Landh. "We don't know how many of these craters there are; we don't even know where they would eventually end up — whether the folds connect to the inner membranes or back to the outer membrane. [And] we don't know *how* the outer membrane is connected to the inner membranes."

Current theories envision the cell membrane as a spherical double layer of fatty lipids — their water-hating ends pointing inward and water-loving ends sticking outward — with various proteins lying at the inner and outer surfaces or pushing through the membrane. These proteins float about slowly in the lipid bilayer.

Landh doesn't dispute this understanding of how the cell membrane works, but he argues that the topology, or overall shape, of cells is almost infinitely more complicated than the traditional view of the cell as a smooth sphere.

Landh describes membranes that fold in and out in three-dimensional shapes so complex that scientists have only recently developed the mathematics to understand their structure — although, he adds, they've been taking pictures of them for the last 35 years.

Not only do cell membranes have a more intricate shape than biologists have ever realized, Landh says, but the shape of the membrane probably affects many cell functions — from protein transport to communications to pressure regulation. Yet, because very few biologists have the mathematical background to visualize these structures as a single, connected membrane, he says, the possible biological implications of these complex membrane shapes are not well understood.

"It's very difficult to picture these structures," Landh admits. "It took me almost 2 years."

Landh became interested in the complex shapes lipids can assume while working on a way to improve the delivery of drugs to specific sites in the body. He became one of the few researchers focusing on the possible three-dimensional arrangements, or phases, of lipids in solution. Different phases can have different shapes. Cubic phases, with lipids arrayed symmetrically in three dimensions, are among the most common.

In 1964, lipid chemist Vittorio Luzzati, now at the National Center for Scientific Research near Paris, proposed that cubic phases might exist in cells. In 1980, Kåre Larsson of the University of Lund in Sweden suggested that the prolamellar body — a structure in plant cells involved in gathering energy from the sun — might share this phase shape.

Both researchers were on the right track, says Landh, but lacked an understanding of the dynamic, continuous interaction of these cubic shapes in the movement and functions of a cell. Landh has dubbed these changing cubic structures "cubic membranes."

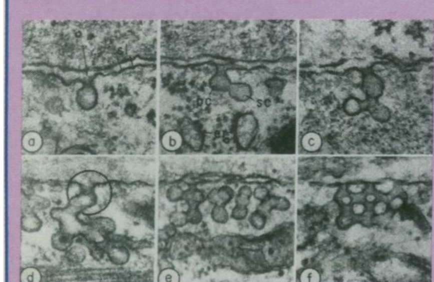
The intricate curves and crevasses Landh imagines come straight from the world of mathematics. The double layer of the cell membrane encloses the curving, continuous shape of what mathematicians call a periodic minimal surface, or PMS.

Like a soap film stretched on a wire frame, these surfaces span the smallest possible area with equal pressure on each side. Many are triply periodic, or capable of extending infinitely in three directions. In three dimensions, minimal surfaces divide spaces efficiently without intersecting — a critical attribute for cells since they can't function without a clear separation between inside and outside.

Landh started wrestling with the three-dimensional structure of cell membranes after a friend showed him pictures of cells taken with electron microscopes. These devices shoot electrons through a sample "slice." Dark patterns appear in areas of more solid material, which scatters the electrons, and lighter patterns mark where more electrons pass through. Some of the patterns made by cell membranes looked like projections of the minimal surfaces that shape cubic phases, Landh noticed.

"At first it made no sense. We were seeing these cubic phases in fish epithelial [skin] cells and then we saw them in scale worms," he says. "We couldn't figure

Cubic shapes show their muscle



H. Ishikawa, Rockefeller University Press

When stimulated, nerve cells release a chemical, called acetylcholine, that triggers muscle contraction. This signal is detected by the sarcolemma, the membrane around a muscle cell, and by transverse tubules, or t-tubules, which extend into the muscle from the sarcolemma. If a nerve cell dies or is physically removed, the acetylcholine signal gets weaker, while the t-tubules grow.

The t-tubules, says Landh, are cubic areas of the sarcolemma and compensate for the lack of signals by amplifying the surface area that can detect signals. Although t-tubules are common in some animals, the presence of t-tubules in humans is often used to diagnose diseases such as muscular dystrophy.

Here, a sequence of electron micrographs shows the beginning of the t-tubule as a pore that successively folds around itself. Any number of pores on the sarcolemma can develop, and eventually an identifiable cubic membrane forms. *— D. Christensen* out why the same exact structure was in such different cells."

He started digging through journals and found a treasure trove: thousands of published pictures of complex membrane structures that had either been ignored or treated as anomalies. They had been given names like "undulating membranes" or "peculiar bodies," and no one understood their cubic, threefold symmetry.

Since then, Landh has been laboriously matching computer projections and electron micrographs. Working backwards to the mathematical PMS, says Landh, should give scientists the most accurate description ever of the three-dimensional shapes of cell membranes.

Cubic membranes aren't simply mathematical constructs, however. They aren't infinitely thin, though they come close (they average about 45 nanometers thick). Each half of the membrane bilayer stretches along one side of the periodic minimal surface. But it matters which side is which because the inner and outer membrane surfaces are sprinkled with different proteins that have different functions. Although mathematical PMSs can repeat infinitely, cubic membranes cannot; they somehow close extending boundaries and meld into more traditional, two-dimensional membranes.

These cubic structures appear most often in active, mature cells, which make and store chemical compounds. Landh has found cubic membranes in the smooth and rough endoplasmic reticulum, the part of the cell that synthesizes fats (lipids) and some proteins; in the inner and outer nuclear envelopes, the membranes that surround the cell's DNA; in the inner mitochondrial membrane, where much of the cell's energy is produced; in lysosomes, small membrane-bound sacs that digest proteins and lipids; and in the plasma membrane, the outer boundary of the cell that controls the transport of molecules in and out.

In fact, he adds, "I haven't found any species that do not have this [cubic] structure in some of [their] cells somewhere," Landh says. "It leads me to believe that every cell goes through the cubic structure at some point in its lifetime."

These complicated structures develop simply. They begin as pores on a flat membrane surface. As lipids move to the area and the membrane grows, the pores fold inward (see drawing at right).

The shape of a cubic membrane depends on the shape of the original membrane from which it forms rather than the shape of its surroundings. To visualize the process, imagine pressing your hand into a sheet of rubber and separating each finger. Assume that the rubber keeps its shape while you go back and shape another "glove" near the beginnings of the original indentation. Without breaking the surface of the rubber sheet, a complicated structure begins to form.

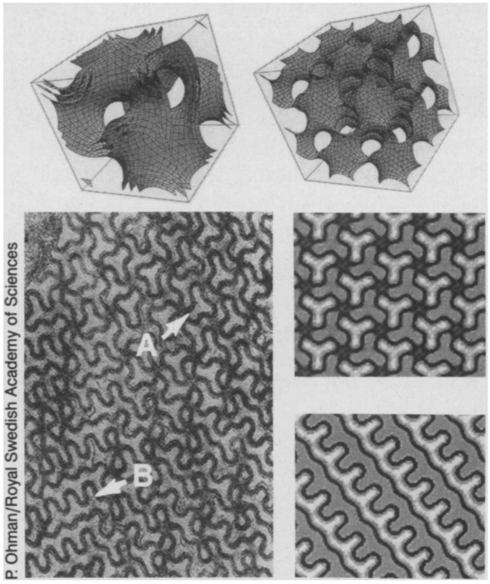
Of course, "there are a lot of ways to get a shape," says University of California, Berkeley, biophysicist George F. Oster. Several biologists have explored other ways that such "undulating membranes" can form, he adds. "Landh proposes it's a natural form of lipid bilayers – and it may well be that biological membranes form in that way – but biologists tend to believe everything is controlled by proteins. Often, [researchers] will find an elegant way [to form cell structures], but cells tend to be dirtier and messier than that. [Landh's] work only points to the fact that there are a great deal of membrane structures that are not well understood."

Cubic membranes "are really a novel idea," says Richard M. Epand, a biochemist at McMaster University in Hamilton, Ontario, who has worked with cubic phases formed outside the cell. "People have always thought of the cell membrane as being arranged in a sheet, and this is a fundamental change in our concept of how a cell is constructed."

Most lipids can be thought of as flat cylinders, he explains, while others resemble cones. If the conelike lipids try to form a sheetlike structure, there will be gaps where water can interact with the lipids' water-hating sides, so the lipids bend to close these gaps. Thus cubic membranes may form only because the lipids that make up the cell membrane can't fit together in a flat sheet, says Epand.

That's possible, says Landh, but he suspects that the complicated shape of cubic membranes is dictated by their function in the cell.

They may help organize the sheer amount of matter in the cell. To some extent, current theory assumes that chemicals simply bump into each other. But cubic membranes, as periodic minimal surfaces, partition space with the maximum ratio of surface to volume. So they may in fact define organelles, such as the endoplasmic reticulum, as they separate space into subspaces.



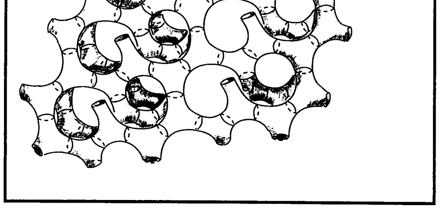
P. Ohman/Royal Swedish Academy of Sciences

A

B

Landh

Clockwise from top left: a periodic minimal surface; repeating units of this PMS (also shown on cover); two-dimensional projections of this PMS (a,b); and similar shapes in an electron micrograph of the smooth endoplasmic reticulum of a river lamprey (a,b).



Landh

***A** sketch showing how cubic membranes can develop from a flat surface.*

"It's an organizational principle," Landh says. "If you build a house you don't want a bedroom in the kitchen or a kitchen in the basement. And you want to organize within these spaces so that you have spices on one shelf and rice on another. The cell is facing the same kind of problem, but it's a dynamic structure and rearranges all the time."

The cell may knock out walls to make larger rooms or build walls to separate different areas, he adds. It's even possible that viruses can induce the formation of cubic membranes, coercing the cell into building the virus its own safe room in which to reproduce.

The cytoskeleton, the protein scaffolding of the cell, may be part of the unknown boundary – the frame for the "soap film" – of cubic membranes. It may run through parts of the cubic membrane or be linked to it at certain areas. "I believe the cytoskeleton and cubic membranes are working in cooperative ways to transport things and change sizes," says Landh. "We've seen evidence of cytoskeletal activity when cubic membranes are forming. Cubic membranes can survive without the cytoskeleton, but when they exist together, they work together."

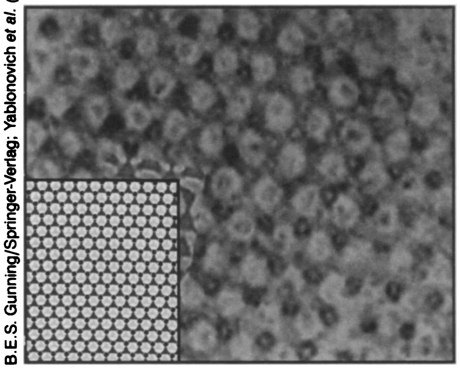
These complex membranes also enable cells to maximize their surface area and therefore their activity. The smooth endoplasmic reticulum – which synthesizes many of the cell's proteins and lipids and needs a large surface area for this activity – is probably a cubic structure formed from the outer nuclear envelope, says Landh. Similarly, cubic structures occur more commonly in the energy-producing mitochondria of hummingbird muscle than in the mitochondria of less active human muscle.

Cubic membranes may help the cell establish and control different chemical concentrations, or gradients, by transporting chemicals across the bilayers. A chemical gradient can act as a wind, pushing the cubic membrane away from the minimal surface. This instantaneously changes the shape and pressure of the subspaces the membrane divides.

In addition, the cell can make itself smaller by folding more lipids into cubic structures, says Landh, and then increase its size by unfolding.

Because PMSs increase the area of contact between two physically separated subspaces, they may increase transport and communication speeds between two such spaces. If the structure is made of several bilayers lying parallel to the PMS, Landh proposes, it might even serve as a membrane-based "communications center," where membranes coming from the endoplasmic reticulum or the nuclear envelope or the cell surface can interact with each other.

Landh "really makes a convincing case that will make a lot of biologists sit up and take notice," says physical chemist John M. Seddon of the Imperial College of Science, Technology, and Medicine in London. "The most important thing he's done is to go to the biological literature and analyze [their own pictures]. Often, we publish in the same journals, but [biologists] tend not to look at [our work]."



B.E.S. Gunning/Springer-Verlag; Yablonovich et al. (inset)

An electron micrograph of the prolamellar body and (inset) *a cross-section of a lab-made photonic crystal.*

Biologists have not traditionally considered the shape of membranes as important as the components of the membranes. "It's a wonderful idea he has, though it's perhaps a little early to say the significance of it," says chemist Luzzati. "But when you are facing this wealth of results, there must be something behind it."

Much of Landh's work so far has been theoretical, but he is aggressively pursuing some of its implications. For example, while reading an article on photonic crystals (SN: 11/2/91, p.277), he noticed that their shape resembles computer projections of the prolamellar body, which develops in plants grown in the dark.

Although these two structures have different origins, Landh hypothesizes on the basis of their similar shape that they both trap photons. After the prolamellar body traps enough photons, or particles of light, it changes shape and photosynthesis can begin.

What's more, Landh adds, the shape of the prolamellar body does not seem to vary between species, further implying that its purpose depends on its shape.

Piece by piece, Landh is trying to put together a picture of what these cubic membranes – which can occupy up to half the volume of a cell – actually do. "Since the shape of an object tells us something about the forces that molded it," he says, "the shape itself has consequences for how the object interacts with the environment."

Landh figures he has enough to keep himself occupied for a while. "Sometimes I feel as if I am the only one who understands what I do," he sighs. "But I hope it's one day going to change the textbook teaching of cell structure." □