My Life on a Raft
When I was 11 years old, I stood with my brother outside the Institute of Advanced Study in Princeton waiting for Albert Einstein to come to work so that I could take his picture. My father, a professor of physics at the University of Helsinki, was spending a sabbatical at the Institute. There was Einstein, on the picture that still hangs in my office in Dresden, with his umbrella as always, even though the sun is shining.

I saw several famous physicists in our home in Helsinki. My dream was to become a physicist myself. But fortunately my father, a farmer's son, was a practical man and when I asked him for advice concerning my career choice, he pointed out to me "Kai, I think you are not up to it. Why don't you study medicine instead? Then you can do research and if you don't like it, you at least have a profession!"

I followed his advice and have never regretted it. I began to study medicine, but inspired by the physical laws that define molecular interactions, I soon moved into biochemistry research. My
MD/PhD research project in 1964 was concerned with vitamin B12 absorption. I purified intrinsic factor, the protein required for vitamin B12 uptake from the intestine, from a pool of 30 liters of human gastric juice. The real mystery was how the protein binding could facilitate vitamin B12 passage across the intestinal cell membranes. This work started my obsession with cell membranes that has persisted to this day.

During my postdoc in New York at the Rockefeller University, I met Finnish virologist Leevi Kääriäinen, who told me about the virus that he was studying, the Semliki Forest virus. This was exactly what I had been looking for—a simple membrane model. This virus stole its envelope from the plasma membrane of the host cell, in the process excluding all host membrane proteins and replacing them with the viral spike glycoprotein. After coming back to Helsinki, Leevi and I were joined by a lipid biochemist, Ossi Renkonen, forming a troika to study this virus membrane. What was absolutely unique at that time was that we included both lipids and proteins in the analysis. We demonstrated that the virus membrane contained the lipids of the host cell plasma membrane and embedded the viral spike protein in the lipid membrane so that the virus could exit from the host cell. The study not only gave us insight into basic viral biology, but offered an experimental system to study plasma membrane biogenesis in general.

That research gave me the entrace ticket to an international career. In 1975, I was invited by Sir John Kendrew to join the newly founded European Molecular Biology Laboratory (EMBL) in Heidelberg. We again formed a troika, this time with my two graduate students, Henrik Garoff and Ari Helenius (read Helenius’ account in our October 2008 issue). We continued the studies on Semliki Forest virus, now using the virus as a tool to study endocytosis and exocytosis in the host cell. This research became a textbook classic and explained how a membrane virus gets into and out of a host cell.

I left Semliki Forest virus to Ari and Henrik, and turned to a new experimental system—one with unique features that I thought could give us a better picture of how the membrane functioned. Some differentiated cells, like intestinal or kidney cells, are polarized. This means that they have two plasma membrane domains, apical and basolateral, with different proteins and different properties. How could a cell with freely moving lipids divide its surface and direct proteins—which were continually replenished and recycled—to the right place on the cell's surface?

The model that I was looking for to test this question was provided by the now famous epithelial MDCK cells that Enrique Rodriguez-Boulan and David Sabatini from New York University had shown to display polarized budding of the membrane trafficking process—a totally unknown area at that time.

I geared up my laboratory for MDCK work and was joined by two fantastic postdocs, Karl Matlin and Gerrit van Meer. Karl was to study protein sorting and Gerrit would go for the lipids. However, setting up the cell cultures we discovered a major problem. We were growing the MDCK cells on plastic supports as Rodriguez-Boulan and Sabatini had done, but with precise assays we found that the cells were only partially polarizing. This meant that no quantitative biochemistry could be done, which was necessary if we wanted to understand the polarization process molecularly. I thought that I had embarked on a path leading nowhere. What a misery!

But then we struck gold. When we tried to grow the epithelial cells on porous nitrocellulose filters instead of plastic

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Lipid Raft Traffic

The components of a lipid raft, produced in the endoplasmic reticulum, begin to coalesce in the trans Golgi network. Raft membranes are enriched for cholesterol and sphingolipids and also contain clustering proteins and trans membrane proteins. Raft vesicles extruded from the Golgi traffic to the surface where they can separate into smaller raft compartments.

polarized cell surface and demonstrated that a fluorescently labeled glycolipid was delivered with higher efficiency from the trans Golgi to the apical, than to the basolateral membrane. For the first time we had shown that polarized delivery of both proteins and lipids is initiated at the Golgi complex.

Pieces of the puzzle had come together: Not only proteins, but different classes of lipids were sorted at different concentrations to the apical or basolateral side of a polarized cell, and they were kept from mingling by tight junctions that created a belt around the cell and formed a gated seal between neighboring cells. We had discovered that this sorting occurred in the layer of the Golgi apparatus that was furthest from the endoplasmic reticulum. We named it the trans Golgi network. In 1988, we published an article postulating that glycolipids and apical proteins were linked to each other within the trans Golgi network. Together they would form transport carriers for delivery to the apical membrane. The concept that specific lipids called sphingolipids and proteins would associate with one another to function in membrane trafficking was totally new, and formed the basis of the raft concept that came to dominate my research.

Ten years later, with Elina Ikonen from the National Public Health Institute in Helsinki, I brought together all of the elements of the raft concept in a review in Nature in 1997. It drew from our observation of sphingolipid-protein assemblies in the Golgi as well as from other sources. Biophysicists using simple lipid model systems had demonstrated that cholesterol plays a key role in regulating how lipids within membranes separate...
into phases, or rafts, rather than diffusing evenly across the surface. We coined the term "raft" for the assemblies of specific lipids, proteins, and cholesterol, and summarized the evidence for their role as platforms for membrane trafficking, signaling and polarization. We proposed three major tenets: 1) the lipid bilayer was not just a homogenous solvent or carrier for membrane proteins, but that the make-up of the lipids regulates lateral cell membrane heterogeneity; 2) rafts are not fixed in size but have the capability to coalesce to form larger functional rafts with scaffolding proteins holding them together; and 3) rafts serve to dynamically compartmentalize the membrane into different "reaction chambers" that allow completely independent processes to occur side by side.

It was difficult to get this review published. One reviewer was vehemently against acceptance, stating that these ideas would set the field back for at least a few thousand person-years. I felt almost proud of my commanding influence. Fortunately, editor Annette Thomas overruled the objections.

One of the biggest problems of studying the molecular interaction of lipid membranes is that they are fickle and susceptible to the slightest changes. When using different microscopic methods on membranes in living cells, researchers have had to contend with the fact that, akin to the Heisenberg's uncertainty principle, we can change the bilayer simply by observing it. For example, biochemical analysis requires that the membrane be solubilized before one can analyze its constituents by the usual analytical tools. This implies breaking many of the interactions that define membrane function. Nevertheless, technological advances have allowed the field to pick up speed in recent years.

In 2001, I moved from EMBL to found a new Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden. With Joe Howard, Tony Hyman, Wieland Huttner, and Marino Zerial—a great team—as directors we have managed to build up a research environment that has attracted worldwide attention. One major focus of MPI-CBG is membrane research, a demanding area that depends on technological innovation. One approach we have taken is the development of lipidomics technology based on modern mass spectrometry that can quantitatively sample the thousands of lipid species making up our cell membranes. Another is imaging. This area has undergone a revolution in recent years and we have tried to capture it within our superb core facilities. A third is membrane biochemistry, including new methods to reconstitute membrane activities in the test tube, and technology to purify transport vesicles. This has allowed us to isolate a transport carrier involved in the raft pathway from the trans Golgi network to the cell surface. Together with Andrej Shevchenko we

Raft Targeting Drugs

We have developed a therapeutic approach that targets raft-bound proteins. The drug inhibits the activity of β-secretase, an enzyme that activates beta-amyloid, which plays a major role in Alzheimer's disease. We designed the inhibitor with a sterol tail that is chemically attracted to cholesterol rich rafts, where it can inhibit amyloid cleavage directly at the vesicle.
have characterized the lipidome and shown that raft lipids are enriched in the carrier vesicles.

When the raft concept was introduced, the idea that a multicomponent cell membrane might contain phases composed of different concentrations and combinations of lipids—that is, phase separation—was met with outright skepticism. Our lab recently demonstrated that lipid phases can indeed separate out within a plasma membrane. Daniel Lingwood developed a method to blow up the plasma membrane of A431 cells into “balloons” similar to giant unilamellar vesicles produced from simple lipid mixtures. These plasma membrane balloons, which contain thousands of protein and lipid species, separate into two types of micrometer-scale phases at 37°C. This separation is cholesterol dependent. One phase is enriched in raft proteins, whereas the other includes non-raft proteins. It was a totally unexpected finding, highlighting the inherent capability of the plasma membrane for phase separation, and raising questions about the evolutionary origins of phase separation.

It is my contention that a phase-separating capability was introduced early in evolution. It would have given primordial cells an easy way to generate organization within membranes. Since then, evolutionary pressure would have developed the complexity of this organizing principle, reflected in the capability for phase segregation. The Gibbs phase rule, proposed in the 1870s, states that the number of different phases should be close to the number of chemically independent components in the system. In an artificial three-component lipid system set up in the lab, two phases can be seen, as predicted by the rule. Actual plasma membranes include hundreds of different chemical components. However, instead of having hundreds of possible phases in our plasma membrane balloons, we only observe two! Membrane lipid and protein species appear to have coevolved to behave as a collective, single entity. This suggests that we should be able to unravel the design principles of the heterogeneity underlying the raft concept. In studying collective behavior of this type, membrane research is showing the way for other areas of biology in which we ultimately have to come to grips with collectives, not simply with protein A interacting with protein B.

Membrane biology has gained prominence in basic biology and disease research. Highly pathogenic membrane viruses such as influenza and HIV employ rafts to exit from the cell by scaffolding a raft domain around their nucleocapsids when they exit from the host-cell plasma membrane. And in Alzheimer’s disease, the cleavage of the amyloid precursor protein (APP) into β-amyloid takes place in specific raft platforms, localized intracellularly in endosomes (see figure on p. 28).

We at MPI-CBG, with the University of Technology Dresden, have launched JADO Technologies, a biotech company focusing on developing lipopholic small molecules for inhibiting membrane raft targets. While the company is currently focused on specifically targeted raft inhibitors for applications to allergy, we have been investigating a β-amyloid raft inhibitor. To deliver the inhibitor to its cellular target, we have synthesized a membrane-anchored version of the inhibitor by linking it to a sterol moiety that embeds into a lipid membrane. The membrane-bound inhibitor is routed to the rafts in the endosomes, where it inhibits β-secretase, efficiently preventing the production of amyloid. The challenge now is to find ways to overcome the blood–brain barrier, because it is in the brain that the inhibitor has to do its job.

The pharmaceutical industry has so far mostly avoided the membrane bilayer itself as a therapeutic target. If the development of lipopholic and raftpholic drugs were possible, then a whole new field of pharmaceutical intervention would open up. Being trained as an MD, it would be tremendous if my life in rafts contributed to moving basic membrane research from the bench to the bedside.

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References:

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