Membranes, viruses, detergents, and endosomes

Ari Helenius
Institute of Biochemistry, ETH Zurich, CH-8092 Zurich, Switzerland

ABSTRACT The fluid mosaic model for biological membranes was formulated 40 years ago. Ten years later endosomes were discovered as important prelysosomal organelles. At the outset of my research career, I was fortunate to witness both these turning points in biochemistry and cell biology from close up, and to participate in some of the studies. In this short essay, I will describe how this came about, and also try to provide some background as to the general starting situation in those not so distant pioneering years of membrane biology.

INTRODUCTION My introduction to research occurred in the summer of 1968, when, as a third-year undergraduate student of biochemistry, I started to work with Kai Simons in the Department of Serology and Bacteriology at the University of Helsinki. This would likely not have happened had he not been the older brother of my girlfriend, Majlen, who is now my wife of 44 years. Having recently returned from postdoctoral study at Rockefeller University in New York, Kai had just started as a group leader and needed “hands.” As it turned out, Kai and I were to work together for the next 13 years, and it was he who most strongly influenced my development as a scientist. Well-informed, enthusiastic, generous, thoughtful, forward-looking, and ambitious, he provided unlimited advice and support. He inspired me—and many others over the years—to explore new ideas and to make the most of our talents.

Kai was studying lipid–protein interactions using human serum lipoproteins as models. Little was known about lipid–protein interactions and membranes at this time. The literature was extremely confusing, and methodological tools were blunt. The SDS–PAGE system to separate denatured proteins according to molecular weight, which revolutionized work on proteins, was just making its debut.

The prevailing view was that membranes provided solid, rigid, waxy walls between compartments. There was no commonly accepted model for their structure. While few doubted that phospholipids could spontaneously organize themselves as bilayers in water, it was not generally accepted that bilayers represented the basic structure of cellular membranes (Stoeckenius and Engelman, 1969). Nor was it clear where the proteins that make up half or more of the mass of most membranes were located. In one model, they were placed on each side of a lipid bilayer, attached to phospholipid head groups. A competing model proposed that membranes were composed of lipoprotein complexes associated side by side, like bricks in a wall. For a while, it was even proposed that all membranes contained the same “structural protein,” a hydrophobic miniprotein. None of these models made much sense functionally, but this was of little concern in this early stage of membrane research.

That the lipid bilayer formed the basis of biological membranes and that membranes were fluid and dynamic became generally accepted in the early 1970s. The emerging consensus was captured by S.J. Singer and G.L. Nicolson in the well-known model that still dominates our thinking about biological membranes: a fluid lipid bilayer with proteins either embedded or adsorbed to the surface (Singer and Nicolson, 1972). The realization that proteins integral to the bilayer are amphiphilic (i.e., that they have distinct hydrophilic and hydrophobic surfaces) and can span the bilayer was a huge step forward. It now became possible to employ a protein-based
My own work focused on the effect of detergents on membranes and lipid–protein interactions. Building on the existing surfactant and colloid literature and the work of Charles Tanford on thermodynamics of membranes and detergent binding to soluble proteins, my PhD thesis presented a conceptual and practical framework for the use of detergents in the solubilization, delipidation, purification, biochemical analysis, and reconstitution of integral membrane proteins (Helenius and Simons, 1975). We found that solubilization—the sudden clearing of a turbid membrane suspension—represented a phase transition from the detergent-saturated lipid bilayer to lipid-saturated mixed micelles. When enough detergent was added, the lipids that covered the hydrophobic surfaces of integral membrane proteins were replaced by a detergent micelle.

The membrane used in our solubilization studies was the envelope membrane of a simple animal virus, Semliki Forest virus (SFV). Because of its simplicity and homogeneity, it was ideally suited for quantitative analysis. We were introduced to SFV by Leevi Kääriäinen, a virologist working one floor above us. Together with Ossi Renvall, a lipid and carbohydrate chemist, Leevi was one of my most important coaches and mentors in these early years.

The work on the virus and its membrane opened the door to international science. In 1975, Kai Simons, Henrik Garoff, Hilkka Virtanen (Kai’s technician for many years), and I joined the newly founded European Molecular Biology Laboratory (EMBL) in Heidelberg. For me, it was time to leave the detergents and all the sudsy solutions with which it was so hard to work. After reading a review about virus receptors by Karl Lonberg-Holm and Lennart Philipson (Lonberg-Holm and Philipson, 1974), I decided to take on the mystery of host-cell entry using SFV, our household virus. This meant that I had to learn something about cells, which we had previously viewed merely as a means to replicate viruses.

Using electron microscopy, Jürgen Kartenbeck, a cell biologist, friend, and collaborator for years to come, saw SFV in coated inden- tations of the plasma membrane and in a variety of cytoplasmic vacuoles. We found that lysosomotropic weak bases, such as ammonium chloride, inhibited infection but did nothing to prevent endocytosis and accumulation of viruses in endocytic organelles. For a long time, we seemed to make no real headway. Fortunately, young group leaders at EMBL, including Bernhard Dobberstein, Daniel Louvard, and Graham Warren, had been recruited to start a program in cell biology. From them, we learned new techniques, such as indirect immunofluorescence microscopy, and about what is now called membrane traffic.

The key to SFV entry into host cells turned out to be the drop in pH that the virus experiences in cytoplasmic vacuoles after clathrin-mediated endocytosis. An acid-induced conformational change converts the spike glycoproteins into efficient membrane fusion machines, and the viral envelope fuses with the limiting membrane of endocytic vacuoles from the inside, releasing the capsid into the cytosol (Helenius et al., 1980a). Together with Jürgen Kartenbeck, Erik Fries, Kai Simons, Judith White, Mark Marsh, Karl Matlin, and others, I described how various viruses (SFV, influenza virus, vesicular stomatitis virus, etc.) took advantage of receptor-mediated endocytosis mechanisms and used low pH as the cue for penetration.

**ENDOSOMES**

In contrast with the secretory pathway, endocytosis had not received serious attention at this time. The uptake of pathogens by phagocytosis in macrophages and amoebae was known, and fluid uptake (pinocytosis) had been described. No molecular approaches had yet been used. Two important observations in the late 1970s changed the situation. It was found by Ralph Steinman and coworkers that membrane and fluid were continuously internalized, and the majority was recycled back to the plasma membrane. Furthermore, clathrin and coated vesicles were shown by Keith Porter, and later by Richard Anderson, Michael Brown, and Joe Goldstein, to serve as primary endocytic organelles for internalization of receptor-bound ligands. This brought in the era of receptor-mediated endocytosis, and it was soon recognized that there was a pathway that not only served as a feeder to lysosomes but supported a variety of other interactions with the outside world.

Perhaps one of the most important observations emerging from the virus entry work was that coated vesicles did not ferry the viruses directly to lysosomes but to prelyosomal organelles. In other words, there existed intermediate, acidic organelles between the primary endocytic vesicles and the lysosomes. We gave these organelles the generic name endosomes in anticipation of yet-to-be-discovered functions (Helenius et al., 1980b, 1983; Marsh et al., 1983). Cell biologists studying receptor-mediated endocytosis of physiological ligands were also finding evidence for intermediate organelles. In particular, Ann Hubbard at Yale and Ira Pastan at the National Institutes of Health, whose research groups studied the internalization of asialoglycoproteins and α2-macroglobulin, respectively, concluded that after uptake by clathrin-coated vesicles, ligands passed through electron-lucent vacuoles en route to lysosomes (Wall et al., 1980; Willingham et al., 1980).

In 1981, I moved to Yale School of Medicine, having been invited by George Palade, another great mentor and role model, to join the Section of Cell Biology. My group included Judith White, Mark Marsh, Eva Bolzau, and Jennifer Willsteed from EMBL, and we continued to work on viruses, endosomes, and endocytosis-related topics. Luckily, we were joined by Ira Mellman from Rockefeller University, who had just joined the Yale faculty. He brought with him deep insights into the cell biology of endocytosis and membrane trafficking, and an invaluable spirit of enthusiasm and confidence. Our labs were fused to form the “Mellennius” group. What followed was a great period of companionship, collaboration, and exciting research. The combined group made rapid progress on the role of early and late endosomes in molecular sorting and in virus entry. Having been overshadowed by the more popular and more “populated” secretory pathway, the endocytic pathway established itself in the 1980s as the important and fascinating subject of study that it still is today.

Since then, I have worked with many excellent students and postdocs at Yale and at ETH Zurich. Together, we have entered new areas of research, including membrane fusion, protein folding, quality control in the endoplasmic reticulum, and glycobiology. After several highly rewarding diversions, my current group is again focusing on virus entry and the role of endosomes, using new tools and new virus systems. One basic premise has remained unchanged during all these years: we have relied on viruses to guide our work. Trusting that they understand cell biology better than we do, we simply follow their lead in the hope of exposing secrets buried deep inside cells.
REFERENCES
Helenius A, Kartenbeck J, Simons K, Fries E (1980a). On the entry of Semliki Forest virus into BHK-21 cells. J Cell Biol 84, 404–420.
Helenius A, Marsh M, White J (1980b). The entry of viruses into animal cells. Trends Biochem Sci 5, 104–106.
Helenius A, Mellman I, Wall D, Hubbard A (1983). Endosomes. Trends Biochem Sci 8, 245–250.
Helenius A, Simons K (1975). Solubilization of membranes by detergents. Biochim Biophys Acta 415, 29–79.
Lonberg-Holm K, Philipson L (1974). Early interaction between animal viruses and cells. Monogr Virol 9.
Marsh M, Bolzau E, Helenius A (1983). Penetration of Semliki Forest virus from acidic prelysosomal vacuoles. Cell 32, 931–940.
Singer SJ, Nicolson GL (1972). The fluid mosaic model of the structure of cell membranes. Science 175, 720–731.
Stoeckenius W, Engelman DM (1969). Current models for the structure of biological membranes. J Cell Biol 42, 613–646.
Wall DA, Wilson G, Hubbard AL (1980). The galactose-specific recognition system of mammalian liver: the route of ligand internalization in rat hepatocytes. Cell 21, 79–93.
Willingham MC, Maxfield FR, Pastan I (1980). Receptor-mediated endocytosis of alpha 2-macroglobulin in cultured fibroblasts. J Histochem Cytochem 28, 818–823.