Virus entry: What has pH got to do with it?

Ari Helenius

After several years of working on membrane proteins using the Semliki Forest virus (SFV) as a model, I decided in 1976 to focus my research on the mechanisms by which animal viruses such as SFV enter and infect their host cells. I had just moved from Finland with the lab of Kai Simons to the newly founded European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. I was eager to tackle a challenging problem — and one that did not require work with detergents.

Relying almost exclusively on electron microscopy, previous work on animal virus entry had left the field divided between proponents of direct virus penetration through the plasma membrane of the host cell and those who favoured penetration into the cytosol from intracellular vacuoles after endocytosis. Together with Jürgen Kartenbeck, a colleague in the German Cancer Research Center (where we were temporarily housed), and Erik Fries, a Swedish post-doctoral fellow, we studied what happened when SFV was added to cells in tissue culture. Using electron microscopy, we visualized virus particles binding to the cell surface, becoming internalized by coated vesicles and accumulating in intracellular vacuoles. Biochemical assays with radioactive virus particles demonstrated that endocytosis was rapid and efficient. We confirmed that infection was prevented by weak bases such as ammonium chloride and chloroquine, and showed that this involved inhibition of virus entry. However, these compounds did not inhibit the endocytic uptake of SFV. To our disappointment, we had no evidence of fusion between host and virus membranes.

Ari Helenius is at the Institute of Biochemistry, ETH Zurich, HPM E6.3, 8093 Zurich, Switzerland.
e-mail: ari.helenius@bc.biol.ethz.ch

For almost two years, the virus entry pathway and mechanism of host penetration continued to elude us. However, in the spring of 1978, I had the chance to participate in a Dahlem Conference in Berlin called 'Transport of Macromolecules in Cellular Systems'. It was an opportune time for a meeting on this topic: pathways of vesicle transport were being discovered, receptor-mediated trafficking processes were beginning to be described, and the important role of clathrin-coated vesicles was finally properly appreciated. All the leaders in the membrane cell biology field were present. During a coffee break, I asked William Sly, a pioneer in the trafficking of mannose-6-phosphate receptors, whether he knew what weak bases such as ammonium chloride and chloroquine do to cells. He directed me to a paper by Brian Poole showing that these agents raise the pH in lysosomes.

When Erik Fries and I were discussing the conference in the laboratory the following week, he made a remark that changed everything. He simply asked, "Could pH have something to do with it?" Suddenly, all the pieces of the puzzle fell into a coherent picture. We realized that the acidic pH in lysosomes and other endocytic vacuoles was not only required to optimize the degradative action of acid hydrolases, but could also serve as a 'cue' for viruses to activate their penetration mechanism. Exposure to low pH 'told' the viruses that they had entered a cell and reached the endocytic pathway, and that it was time to activate the penetration machinery. We speculated that low pH induced a change in the spike glycoproteins present on the virus envelope that allowed fusion of the viral envelope from the lumenal side with the limiting membrane of the vacuole. As a result, the viral capsid was released into the cytosol without itself having to cross the hydrophobic barriers formed by a membrane.

Over the following months, we validated all the predictions of our pH hypothesis. For example, we could demonstrate a membrane fusion reaction *in vitro* by simply mixing liposomes and SFV, and briefly dropping the pH to 6 or below. Moreover, when we added acidic medium to cells, we could 'fool' surface-bound viruses into fusing with the plasma membrane; the entry block caused by weak bases was bypassed and the cells became infected.

Joined by Judy White, Mark Marsh and Karl Matlin as postdoctoral fellows in the lab, we extended the acid-activation concept to other viruses. For the influenza A virus, the threshold was a pH-unit lower, and exposure to low pH induced a dramatic, irreversible conformational change in the haemagglutinin glycoprotein. We realized, moreover, that the acidic organelles where viruses were activated were not lysosomes, but pre-lysosomal vacuoles. We called these vacuoles 'endosomes', a name that soon became the general term used for these complicated and dynamic compartments.

Today, we know that most viruses (whether enveloped or not) use endocytic entry mechanisms. For the majority, a drop in pH serves as a trigger for host penetration. Some bacterial toxins also rely on low pH as a cue. Moreover, it is well known that differences in the pH between compartments regulate directional transport of cargo in the endocytic and secretory pathways. Endosomes have attained a visibility and recognition that those of us working on them in the early 1980s could hardly have dreamt of. I have had other turning points along my scientific path, but none of them with quite as many significant consequences as the one involving endosomal entry of SFV and the role of pH.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.