The development of structural virology

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Let me begin with thanks to the Paul Ehrlich Stiftung, for honoring me with this prize, and to Prof. Norrby, for his elegant Laudatio. I would like also to note my pleasure in sharing this award with Prof. Michael Rossmann, whose work has inspired and enabled several generations of structural biologists.

A quotation from Paul Ehrlich, taken from his Nobel lecture, is a good place to start, in explaining to you the nature of the research that has led to this prize. Ehrlich, as you recall, had used chemical dyes and light microscopy to study the differentiation of cells in the immune system. He wrote: *"... ich möchte glauben, dass das, was das Mikroscop uns leisten konnte und geleistet hat, jetzt sich seiner Grenze nähert und dass für ein weiteres Eindringen in das wichtige, alles beherrschende Problem des Zellebens die Anwendung optischer ... Hilfsmittel versagen muss. Gerade jetzt ist die Zeit gekommen, in den feinsten Chemismus des Zellebens einzudringen und den Vollbegriff der Zelle in eine grosse Zahl einzelner bestimmter Partialfunktionen zu zerlegen. Da aber das, was in der Zelle geschieht, im wesentlichen chemischer Art ist and da die Gestaltung chemischer Strukturen ausserhalb der Grenze der Sichtbarkeit gelegen ist, werden wir hier nach anderen Forschungs-methoden uns umsehen müssen. Diese Richtung ist nicht nur zum wirklichen Verständnis der Lebensvorgänge überhaupt von Wichtigkeit, sondern sie ist auch die Grundlage einer wirklich rationellen Verwendung der Arzneistoffe."* This remarkable statement could have been taken as a program for research in Cell Biology and Biochemistry for the entire 20th century.

In 1963, fifty-five years after Ehrlich's lecture, I began, as a student, to think about how I might make my own small contribution to 20th century Biology. Max Perutz and John Kendrew had shown how to visualize the three-dimensional arrangement of atoms in a protein molecule. This was the ultimate dissection of a cell into its working parts. It therefore seemed to me that it was time to begin the climb upwards from the intimate details of O_2 transport and enzymatic catalysis toward the cell as an integrated ensemble of molecular machines. Understanding the atomic organization of simple viruses was clearly a good place to begin. Viruses are elegant simplifications of many aspects of cell biology. They are often devastating pathogens, remarkably difficult to control, either at the level of individual infection or at the level of spread in a population. So their biology is of great importance to human society. And the experimental way forward, toward an atomic-level understanding of their structure, their assembly, their mechanism of cell entry, and their immunological specificity, had already been indicated by the pioneering work of J.D. Bernal, Rosalind Franklin, Don Caspar, Aaron Klug, Ken Holmes and their many co-workers. These investigators had shown that it might just be possible to extend the same method -- the technique of X-ray crystallography so brilliantly exploited by Perutz and Kendrew to understand haemoglobin and myoglobin -- to analyze the atomic details of a virus particle and hence to see how an assemblage of protein subunits and RNA or DNA might form, escape from one cell, enter another cell, and uncoat, delivering its genome into the new host. Michael Rossmann had also pointed the way, in quite a crucial fashion, by demonstrating (with David Blow) that the spectacular symmetry of simple virus particles could be used to make the task much easier.

But where to begin? Genetic engineering and expression of recombinant proteins were 20 years in the future. One had to use biological material that was readily available in large quantities, and the

RNA plant viruses were obvious candidates. Thus, in 1965, while spending a year as a student in the laboratory of Aaron Klug in Cambridge, I wrote to my future PhD mentor, Don Caspar, asking whether I could undertake crystallographic studies of tomato bushy stunt virus for my thesis research.

Needless to say, it took more than a PhD thesis to achieve the goal! Indeed, it was somewhat over 12 years later, in the summer of 1977, that an atomic picture of TBSV finally emerged. The result was at once spectacularly exciting and spectacularly disappointing. Exciting, because one could indeed see how evolution had arrived at the functional architecture of a cell-to-cell RNA delivery system -- the essential function of a virus like TBSV. To quote David Baltimore, "A virologist is among the luckiest of biologists, because he can see into his chosen pet down to the details of all of its molecules....[He] sees how an extreme parasite functions using just the most fundamental aspects of biological behavior." We had extended that statement to include "the details of all of its atoms." But the result was also disappointing, because the complicated specificity of the structure showed that a vast amount of work would be needed to understand how even the simplest of the *human* RNA viruses might assemble and how it might enter a cell and uncoat. Michael Rossmann took the first really important step in that direction in 1984, when he and his co-workers determined the atomic structure of the human common-cold virus (human rhinovirus). He will doubtless tell that story in his talk, but one aspect of the result bears mention already. The atomic structure of the human rhinovirus revealed a quite surprising similarity to that of TBSV (a similarity that I believe Michael Rossmann partly expected -- his intuition on this sort of matter has been remarkable -- but that I had not expected at all). An important consequence was to unify the biology of plant and animal viruses and to tie together the nascent molecular biology of RNA plant viruses with the more mature molecular biology of poliovirus, rhinovirus, and their relatives.

What about application to human disease? Michael Rossmann will probably tell you about therapeutic approaches, based on knowledge of virion structure, that depend on preventing viral attachment or penetration. In their role as delivery vehicles, virus particles, and their surface proteins, undergo dramatic conformational rearrangements in order to accomplish infection. The gyrations of fusion proteins found on enveloped viruses like influenza virus and HIV-1 and revealed by the crystallographic work of my colleague, Don Wiley, are noteworthy examples. Protein conformational changes are good targets for drug-like inhibitors, since simple ligands can selectively stabilize one state or another of a multi-state assembly. Structural understanding is also fundamental to improved vaccine design. Let me cite one recent example from our own work. Three years ago, Xiojiang Chen, then a postdoctoral fellow in our laboratory, determined the structure of the outer-shell protein (known as L1) from human papilloma virus (HPV) type 16 -- a virus that potentiates development of human cervical cancer. An effective HPV vaccine could reduce or eliminate this second most prevalent cancer among women world-wide. To our surprise, the form of HPV16 L1 and the conditions for crystallizing it led to what is in our view an excellent candidate immunogen. We have begun a collaborative effort to explore this possibility. Thus, curiosity about the assembly and stability of this virus -- the fundamental questions that drove our structural research -- and the biochemical manipulations required to get satisfactory crystals led to discoveries directly relevant to vaccine design. Led "by chance" to such discoveries, I might have said -- but I do not believe it is by chance. Structure and conformation, as Ehrlich recognized long before it was known that antibodies were proteins, are essential for specific antigenicity, and the more we understand about these matters, the more we can begin to control and plan vaccine development.

In concluding, I thank my many co-workers and collaborators, who have made all this research possible, and I also thank all of you for joining us in remembering the remarkable lives and work of Paul Ehrlich and Ludwig Darmstaedter.