AARON KLUG AND STRUCTURAL MOLECULAR BIOLOGY

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"Much of the fabric of a cell, including its internal skeleton, is built of arrays of large biological molecules such as proteins and polysaccharides. Similarly, the enzymatic and synthetic machinery within a cell consists primarily of such assemblies, which are sometimes so distinctive in form and function as to deserve the name 'organelles'. The assemblies concerned with replication of the cell and its genetic component involve the nucleic acid polymers: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which form complexes with various sets of proteins. Methods over the last 30 to 40 years- notably X-ray diffraction and electron microscopy - have been developed for investigating these kinds of assemblies, so that precise information about their structures is possible, in cases right down to molecular detail. This enables the study of chemical interactions between structural units constituting these ordered biological systems. Complemented by biochemical studies allowing researchers to follow the path (or paths) by which the systems are assembled out of their constituent units, the information about the assemblies' structures allows us to begin to understand the chemical and physical principles underlying their formation. The key principle that runs through these systems is the notion of specificity, which enables the different constituent molecules to recognize each other and exclude others that do not belong, so that no external instructions are necessary to form the assembly. In other words, the design of an ordered structure is built into the bonding properties of its constituents, so that the system "assembles itself" without the need for a scaffold. I have been privileged to have played a part in laying the foundations of this subject, which is now often called Structural Molecular Biology." (Aaron Klug, Leonardo, 1997).

Prof. Aaron Klug's major scientific accomplishments, achieved together with his collaborators, can be grouped into six areas:

1. The structure and assembly of Tobacco Mosaic Virus (TMV).

The group of Rosalind Franklin mapped out the general outline of the structure of the rod-shaped tobacco mosaic virus (TMV). They showed the structure to be a spiral of RNA, which carries the genetic information encased in a helical array of protein units arranged rather like corn-on-the-cob. (A. Klug, as told to E. Garfield, Current Contents, 1984).

After Franklin's untimely death in 1958, Klug led the group and initiated studies on the assembly of the virus particle out of its constituent protein and RNA. "Klug hypothesized that, as in crystal growth, the slow step was nucleation. He found that preformed disks could serve as nucleation centers, reducing the time of assembly to a few minutes. Crystals of the disk suitable for x-ray analysis were obtained but, because of the larger size of this 34-subunit aggregate, the determination of its structure by crystallographic methods was a formidable technical and analytical problem. After 12 years the structure was solved to atomic resolution. Adjacent to the central hole of the disk there is a gap between the two rings rather like a pair of jaws. The TMV RNA has a specialized region that binds inside a pair of these jaws. In a nucleation mechanism, elucidated by Klug, the disk dislocates into a helical structure after binding a loop of the RNA. Thus, the disk is shown to be an obligatory intermediate in assembly. It initiates assembly of the protective protein coat about RNA, and it is able to reject foreign RNA by failing to bind RNA's that lack the specialized region. Thus, in terms of the principles of virus construction formulated many years previously, the self-assembly of TMV is a self-checking process dependent on the subassembly of protein disks". (Science, 1982, D. L. D. Caspar and D. J. DeRosier.)

"I worked on TMV from 1954, when I joined Rosalind Franklin, to the 1970's, when we were finally able to prove the mechanism of the assembly. Even after many years, this is the most detailed system of its

kind that has been worked out. This was an important achievement, and for me, TMV was my first major scientific adventure". (The Chemical Intelligencer, (CI)), 2000.

2. The architecture of spherical viruses.

Klug moved on to the study of the structure of spherical viruses, where the infective RNA is encased in a shell of protein subunits. He and John Finch showed that both the turnip yellow mosaic virus and poliovirus have shells with five-fold (icosahedral) symmetry. This was significant as it showed, contrary to what was generally then believed, that animal and plant viruses are not fundamentally different. At the same time it raised a conundrum, since the maximum number of protein subunits that can be assembled into a spherical shell with perfect icosahedral symmetry is 60, yet chemical studies showed the number was often some hundreds. Inspired by Buckminster Fuller, Klug and Donald Caspar showed that viruses are 'self-assemblies" built like "quasi-equivalent" geodesic domes. Viral assembly was considered analogous to a crystallization process that seeks to meet the thermodynamic requirement of structural minimal free energy. They removed the existing requirement for exact symmetry in virus structure and showed that certain designs with icosahedral symmetry would allow all the subunits to make nearly equivalent interactions with each other. This allows for viruses to assemble themselves spontaneously without the need for additional "instructions" or "scaffolding." All spherical viruses studied so far have one of the designs predicted by Caspar and Klug.

3. Three Dimensional Image Reconstruction in Electron Microscopy.

Excerpted from the Nobel Prize Press Release (1982): "Aaron Klug, who has been awarded this year's Nobel Prize in chemistry, has developed a method for the structural determination of biologically functional molecular aggregates. His technique is based on an ingenious combination of electron microscopy with principles from diffraction methods… Klug has shown that pictures of biological objects seemingly lacking in contrast often contain a large amount of structural information, which can be made available by a mathematical manipulation of the original picture. His method allows electron microscope pictures of high quality to be obtained without the use of heavy metal stains. In this way changes in the sample are minimized, so that the electron microscope picture at high resolution is a true representation of the original biological structure. The method gives a two-dimensional projection of the sample only, but Klug has shown that a three-dimensional reconstruction of the object can be obtained by collecting pictures in several different directions of projection. The method of Klug makes it possible to determine structures at high resolution of functionally important molecular aggregates." The technique is the forerunner of electron tomography or crystallography. It was also the basis of the principle of the X-ray CAT scanner.

4. The structure of chromatin.

Nobel Prize Press Release (1982): "The DNA-protein complex of cell-nuclei, chromatin, is condensed to chromosomes during cell division. In a given cell, only a part of the genetic message in DNA is transcribed, a fact which must also be related to structural changes in chromatin. Knowledge of chromatin is consequently of great importance for an understanding of the control functions of the cell. Chromatin is too large a molecular aggregate to allow a direct structural determination even by the method of Klug. With his co-workers, Klug has succeeded in breaking down chromatin to fragments which are small enough to be studied by x-ray diffraction and electron microscopy. Klug has then been able to construct a model for the chromosomes based on his knowledge of the structure of the fragment."

There is a hierarchy of levels of folding of the long DNA molecule in a chromosome. The basic structural unit is the nucleosome, in which 160 base pairs of the DNA double helix are wound in two superhelical turns of the helix about a spool formed of fours pairs of histone proteins. In the next level of folding, mediated by a fifth histone, the nucleosomes coil into solenoidal filament, followed by further folding which is less well understood.

5. The structure of Transfer RNA and an RNA enzyme (ribozyme).

In 1974 Klug's group was the first to obtain crystals of a transfer RNA and to determine its structure. Transfer RNA molecules bind to ribosomes and decode the nucleotide sequence of the messenger RNA and so transfer the information into the amino acid sequence of the protein being synthesized. "Francis Crick was in the Lab, and he said we must find out the structure of tRNA, and we did… Our work on the tRNA structure had important consequences for later work in that one of the metals binding to the molecule caused it to act as a metalloenzyme, which cleaved the RNA. This led me later to work on RNA enzymes (ribozymes)... [In the 90's] we began working on a real RNA enzyme, the "hammerhead" ribozyme, and were able to solve its native structure and also capture various stages in the catalyzed reaction by fast freezing". (excerpted from CI). They solved the crystal structure of an all-RNA hammerhead ribozyme and worked out the mechanism for an RNA catalytic cleavage. Artificial ribozymes are presently being studied for therapeutic applications.

6. The discovery of "zinc fingers."

Prof. Klug's present major research interest is in this area. Certain stretches of amino acids can fold independently around a zinc ion, forming modules that came to be called "zinc fingers" because it was used to "grip" the DNA double helix. Since zinc fingers function as independent modules, each binding to three base pairs, fingers with different specificities can be linked together to recognize longer DNA sequences. Proteins engineered on the zinc finger design have been used to target specific DNA sequences and, in combination with activation or repression domains, to switch genes on or off.

Israel and Basic Research:

Prof. Klug is a frequent visitor to Israel. He sits on several academic advisory committees, including that of the Weizmann Institute, the Hebrew University of Jerusalem, and has also chaired a committee of the Israel Academy of Sciences. The last 5-6 years he has devoted efforts in Israel to the Ben-Gurion University of the Negev. He recalls in 1950, as a student in Cambridge, applying for a post at the Weizmann Institute. He was not offered a position.

Klug considers that Israel is scientifically in good shape, more so when one takes into account the country's overall situation. He is a strong proponent of investment in basic research, and points out the many high tech and science based companies today that are a result of the country's investments made some twenty years ago. He mentions the Technion as an example of investment in an institution that has paid back society by providing the basis for high tech industry. At this time, Klug feels that there should be investment in biotechnology, and in chemistry and physics. Basic science is important in its own right. Chemistry and physics should not be viewed as aids to other fields, "Hand-maidens". "Look at my own work", he says, "who could have seen that my work on viruses would lead to the CAT scan?" He takes a dim view of scientists that do not develop applications when they arise in course of "pure" research. "If in the course of your work, you find something that can be applied, then it is not your choice, but your obligation, to exploit it. Spin off companies pay back the public for their investment in basic research".

"Basic research can ultimately yield extraordinary returns to society, but it is difficult to estimate its benefits quantitatively since its results may be used in many directions. Moreover there is often a significant delay between the dissemination of fundamental knowledge and its eventual effect on industrial processes. In the 1996 report on science indicators by the National Science Board of the USA, the delay is estimated at approximately ten years for new knowledge in computer science and engineering and 20 years for new knowledge in science and engineering in general (think of the laser). So, basic investment must be made many years ahead and the investment must be not only in institutions, but above all in people, in human resources. Research may be initiated in programs and by committees, but it takes place in the brains and hands of individuals". (Annual Address of the President of the Royal Society, 1996).

Aaron Klug

Aaron Klug was born in 1926 in Lithuania and from age two grew up in South Africa. He began his academic studies as a medical student, but switched to natural sciences after courses in Physiology, Biochemistry and Histology. He earned the B.Sc. degree (1945) in Chemistry, Physics and Mathematics from the University of Witwatersrand, the M.Sc. degree (1946) from the University of Cape Town and the Ph.D. (1952) in Physics from the University of Cambridge. From 1954 until her death in 1958, he worked with Rosalind Franklin at Birkbeck College, London, on the structure of TMV. There he also began his studies of spherical viruses, including several plant viruses and polio virus. In 1962, he joined the MRC laboratory of Molecular Biology in Cambridge, became joint head of the Structural Studies Division in 1978 and was Director of the Laboratory from 1986 to 1996. He was responsible for spinning off three biotech companies, based on the research in the Laboratory.

During that time he also encouraged genome sequencing, first by John Sulston of the nematode worm C. Elegans, which led the way to sequencing the human genome (Anniversary address of the President of the Royal Society, 2000). The Sanger Centre in Cambridge, a spin-off of the MRC laboratory, was responsible for one third of the human genome. Klug now continues as a member of staff, leading a research group on gene expression. He was President of the Royal Society from 1995-2000. Aaron Klug was honored by the award of the Nobel Prize for Chemistry in 1982. He is a member of the Order of Merit of the U. K, whose membership is limited to 24, a Foreign Associate of the National Academy of Sciences of the USA and of the French Academy of Sciences.

Prof. Klug offers the following advice to the student interested in science: "Follow your instincts. Do what you like doing. Don't attempt the impossible. Try to do something realistic and worthwhile. Don't necessarily go into fashionable subjects. Start the work, even if you are uncertain how to finish it. But remember, if you follow this advice, be aware of the risks that you run". Klug recalls Pirkei Avot (Ethics of the Fathers) 2:21. "It is not your obligation to complete the task, but neither are you free to desist from it."