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NEWS

NOBEL PRIZE IN CHEMISTRY 2009

The Nobel prize in Chemistry 2009 has been awarded for 'the studies of the structure and function of the ribosome' to **Ada E. Yonath** from the Weismann Institute of Science, Rehovot, Isreal, **Thomas A. Steitz** from the Howard Hughes Medical Institute, Yale, USA, and **Venkatraman Ramakrishnan** from the MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

In 1859, Charles Darwin published the theory of evolution. It is based on the assumption that an organism's properties are hereditary, however, random changes occur in it. Successful changes that increase the chances of survival of the given organism are carried forward into future generations. Once the scientific community could understand the Darwin's concept, some new questions arose: what exactly is being transferred over generations? Where do the random changes occur? And how can they manifest themselves in a living organism?

The 2009 Nobel Prize in Chemistry is the third in a series of prizes that show how Darwin's theories actually function at the molecular level. The trilogy of prizes began with one of the most famous Nobel Prizes of all, that of 1962, when James Watson, Francis Crick and Maurice Wilkins were recognized for their contributions toward deciphering the structure of DNA. The second prize in the trilogy was awarded in 2006 to Roger D. Kornberg for X-ray structures that explicate how information is copied to the messenger RNA (mRNA) molecule. Ribosomes are large (2.5 MDa) molecules that are present in cells of all living organisms and they read the information in messenger RNA, and produce proteins based upon the information. It is during this process, when DNA-RNA language becomes protein language, and a cell manifests its full complexity. The three Nobel Prize Laureates in Chemistry for 2009, **Ada E. Yonath**, **Thomas A. Steitz** and **Venkatraman Ramakrishnan**, have been rewarded for mapping the ribosome.

Let us begin with the mystery that fascinated chemists and biologists during the middle of the twentieth century: how does life function from a chemical point of view?

At the beginning of the 1940s, scientists knew that hereditary traits were carried by chromosomes. Chromosomes are comprised of nucleic acids (DNA) and proteins. The majority of the scientific community thought that proteins were the carriers of hereditary traits, since they are more complex than DNA. The year of 1944, however, saw the return of the DNA molecule by famous Avery-MacLeod-McCarty experiment which rendered non-virulent bacteria into virulent after DNA transfer. In February 1953, James Watson and Francis Crick at the Cavendish Laboratory at Cambridge University, UK, made the scientific community realize that the genetic code is contained within the nucleotide sequences on each of the strands. ATTGCCAT represents something com-pletely different from GCGTATAG. Scientists realized that the sequence of nucleotides controls the sequence of amino acids in proteins. But the question remained: How?

Thus, 100 years after Darwin elaborated his theory of evolution, scientists had identified DNA as the molecule that carried hereditary traits. The sequence of nucleotides in DNA controls the sequence of amino acids in proteins, which are produced by ribosomes in the cytoplasm. But what is the link between DNA and the ribosomes as they are located on different sides of the nuclear envelope and have no direct physical contact with one another?

Often a ground-breaking discovery comes from a pioneer who investigates new uncharted territory. In this case, that pioneer was Ada Yonath. At the end of the 1970s, she decided to try to generate X-ray crystallographic structure of ribosomes, however, it was indeed a challenging task. In X-ray crystallography, scientists aim X-rays towards a crystal of, for example, a protein. When the rays hit the crystal's atoms they are scattered. On the other side of the crystal, scientists register how the rays have spread out. Previously, this was achieved by using photographic film, which was

blackened by the rays. Today one uses CCD detectors, which can be found in digital cameras (a technology that brought Nobel Prize 2009 in Physics to **Charles K. Kao, Willard S. Boyle and George E. Smith**). By analyzing the pattern of dots, scientists can predict how the atoms are positioned in a protein. However, for the procedure to work successfully, the crystal has to be almost perfect and the molecules need to form a precise pattern which is repeated over and over again. Obtaining high quality crystals from a protein is a hard task, and the larger the protein complex, the harder the task. Therefore, many scientists were indeed skeptical of Ada Yonath's vision. The ribosome is one of the most complicated protein/RNA complexes. It is divided into two parts: the small subunit and the large subunit. Each of the subunits consists of thousands of nucleotides and thousands of amino acids, which in turn consist of hundreds of thousands of atoms. Ada Yonath wanted to establish the exact location of each and every one of these atoms in the ribosome.

When Ada Yonath decided to crystallize the ribosome, she chose to work with bacteria living under harsh conditions. *Geobacillus stearothermophilus* can live in warm springs and survive in temperatures up to 75 °C. Ada Yonath's assumption was that, in order to survive in this temperature range, the ribosome has to be extremely stable and thus would form better crystals. In 1980, she had already managed to generate the first three-dimensional crystals of the ribosome's large subunit. This was a great achievement, although the crystals were far from perfect. It actually took another 20 years of hard work before Ada Yonath managed to generate an image of the ribosome where she could determine the location of each atom. She tried many new things. For instance, she stabilized the crystals by freezing them in liquid nitrogen at -196 °C. She also tried to crystallize ribosomes from other resilient micro-organisms. One of them was – the salt-loving *Haloarcula marismortui* that lives in the Dead Sea. Step by step, Ada Yonath got closer to the goal. Eventually, it was realized that the ribosome's atomic structure could be mapped, and more scientists joined in the challenging journey. Among them were Thomas Steitz and Venkatraman Ramakrishnan.

At the beginning of the 1990s, Ada Yonath's crystals had sufficient quality. The pattern of black dots was detailed enough to determine the location of the atoms in the ribosome crystal. Nevertheless, there remained a considerable obstacle, however. It was the "phase problem" of X-ray crystallography. In order to determine a structure from the pattern of black dots, scientists needed to know the "phase angle" for each and every dot. This mathematical information is related to the location of the atoms in the crystal. A trick that is frequently employed by scientists in order to determine phase angles is to soak the crystal in heavy atoms, e.g. mercury. The heavy atoms attach to the surface of the crystals of ribosomes. By comparing the dotted patterns from crystals with and without heavy atoms, scientists can establish the phase angle. However, the ribosomes are so large that too many heavy atoms are attached to the ribosome, and it was difficult to immediately determine the phase angle. Additional information was therefore needed in order to solve the phase problem. It was Thomas Steitz who finally solved the problem. He used images of the ribosome, generated by Joachim Frank, a specialist in electron microscopy. With the help of those images, Thomas Steitz could find out how the ribosomes were oriented and located within the crystal but the resolution did not allow him to see individual atoms. This information, together with the information from the heavy atoms, finally yielded the phase angle.

In 1998, Thomas Steitz published the first crystal structure of the ribosome's large subunit. It resembled a dim photograph, and had a resolution of 9 Ångström. It was not possible to see individual atoms, but one could detect the ribosome's long RNA molecules. This was a big leap forward. Now that the phase problem was finally resolved, all that remained was to improve the crystals and collect more data in order to increase the sharpness of the image. But the question still persisted: How do ribosomes catalyze peptide bond formation? Using high resolution X ray crystallography and molecular computational methods Steitz and coworkers demonstrated that specific residues in ribosomal RNA as well as water molecule were responsible for the network of H-bond formation that facilitated ribosome catalyzed peptide bond formation.

The role of the large subunit in the ribosome is primarily to synthesize new protein. It triggers the peptide bond formation between the amino acids. To obtain a step-by-step image of the chemical reaction is very difficult, as it occurs at the atomic level and at a daunting speed. In a single ribosome, about 20 peptide bonds can be formed every second. Thomas Steitz, however, has managed to freeze different moments of the chemical reaction. He has crystallized the large subunit with molecules resembling those that are involved in peptide bond formation. With the help of these structures, scientists have been able to determine which of the ribosome's atoms are important to the reaction, and how the reaction occurs.

This year's Nobel Laureates reached the finishing line almost simultaneously. In August and September 2000, they published crystal structures with resolutions that allowed interpretation of the atomic locations. Thomas Steitz managed to obtain the structure of the large subunit from *Haloarcula marismortui*. Ada Yonath and Venkatraman Ramakrishnan obtained the structure of the small subunit from *Thermus thermophilus*. Thus it was possible to map ribosome functionality at the most basic, atomic level.

An outstanding property of the ribosome, that fascinated scientists for a long time, is that it seldom makes any error when it translates DNA-RNA language into protein language. If an amino acid is incorrectly incorporated, the protein can entirely lose its function, or perhaps even worse, begin to function differently. For the correct amino acid to be selected depends primarily on the base pairs formed between tRNA and mRNA. However, this pairing process is not sufficient to explain the ribosome's precision. Venkatraman Ramakrishnan's crystal structures of the ribosome's small subunit have been crucial for the understanding of how the ribosome achieves its precision. He identified something that could be described as a molecular ruler. Nucleotides in the small subunit's rRNA measure the distance between the codon in mRNA and the anticodon in tRNA. If the distance is incorrect, the tRNA molecule falls off the ribosome. Using the ruler twice, the ribosome double-checks that everything is correct. This ensures that errors only occur about once per 100 000 amino acids.

The Laureates of the 2009 Nobel Prize in Chemistry have forged an understanding at the atomic level of how nature can transform something as simple as a four letter code into some-thing as complicated as life itself – just as James Watson predicted in 1964. Research driven by curiosity can also, as happened so many times before, be of practical use. This time it proves useful in the search for new antibiotics.

Today, humans have an arsenal of different antibiotics which can be used in the fight against disease-generating bacteria. Many of these antibiotics kill bacteria by blocking the functions of their ribosome. However, bacteria have become resistant to most of these drugs at an ominous rate. This year's three Nobel Laureates in Chemistry have produced structures that show how different antibiotics bind to the ribosome.

Knowing the atomic structure of the ribosome can be used to develop new antibiotics against multiresistant bacteria.The understanding of the ribosome's structure and function is of great and immediate use to humanity. The discoveries that **Ada Yonath**, **Thomas Steitz** and **Venkatraman Ramakrishnan** have made, are important both for the understanding of how life's core processes function, and in order to save lives.

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