THE 2009 NOBEL PRIZE IN CHEMISTRY: FOR STUDIES OF THE STRUCTURE AND FUNCTION OF THE RIBOSOME

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The whole scientific community around the world waits for the day when years of hard work and brain storming research are honoured with the highest prize founded in the name of Sir Alfred Nobel. This year's Nobel Prize in Chemistry (2009) was shared by three scientists Venkataraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath. They have shown what the ribosome looks like and how it functions at the atomic level. The Swedish Academy of Sciences agreed to bestow this honour of their research work on understanding the central dogma of protein synthesis. Using X-ray crystallography they established the 3D structure of protein synthesis machinery i.e. Ribosome. The ribosome reads the information from the mRNA and translates it into long chains of amino acids (polypeptide) and these molecules form proteins of various complex functions depending on its post-translational modifications. The trilogy of Nobel prizes regarding the central dogma, started with James Watson, Francis Crick and Maurice Wilkins elucidating, the atomic model of the double stranded DNA molecules in 1962. DNA is present in every cell of all organisms except for retro- viruses, even it is considered as the blueprint of life. As the activity of any process in cellular functions finally depends on proteins, in recent science, transcription and translation processes are considered more important than replication. Thus the work of Roger D. Kornberg on RNA synthesis in 2006 is also an important milestone. Remarkably X-ray crystallography played a significant role for all of the above three important research works.[20] One of the recent issues in Current Science, explained briefly about all the Nobel Laureates in 2009.[1] In this regard, we thought it would be a good idea to focus on Chemistry due to the involvement of an Indian-American Scientist Dr Ramakrishnan.

Indian Perspective of Nobel in Science

It is a matter of great honour and respects for our country that Nobel Prize in Chemistry 2009 is being shared by an Indian-American scientist Dr. Ramakrishnan with two other elite scientists from USA and Israel. Dr. Ramakrishnan is the first Indian born scientist, to receive Nobel Prize in chemistry. Thus he joins the other eminent Indians like Dr. Hargobind Khorana (Nobel prize in medicine for his pioneer work for interpretation of genetic code in 1968)[10], Dr. S. Chandrasekhar (Nobel in Physics for his ground breaking work on theoretical structure and evolution of stars in 1983)[4], although their award-winning works were carried out in foreign soil. In this regard, Dr. C. V. Raman is the only Indian scientist who received this greatest honour in science in 1930 (due to his novel work, popularly known as Raman spectra, on 28th February, 1928) working in an Indian Laboratory (Indian Association for Cultivation of Science, Kolkata) with limited facility. India celebrates every year their national science day on 28th February, to commemorate his contribution in Indian science. Due to the gradual improvement of science, right now, we have world class scientific facilities in India. Indian people are eagerly waiting to get more noble laureates directly from Indian soil.

A few words about the 2009 Nobel laureates in Chemistry

Dr. Ramakrishnan was born in the year 1952 in Chidambaram, Cuddalore District of Tamil Nadu, India. He graduated in Physics from Maharaja Sayajirao University, Baroda (MSU). After Graduation he moved to USA, where he completed his Ph.D. in Physics from Ohio University in 1976. Later he studied Biology as a Graduate Student at the University of California, San Diego, USA. Followed by his graduation in Biology; he started his postdoctoral work at the Department of Chemistry, Yale University with Dr. Peter Moore. There he first started working on Ribosomal Crystallography. He furthered his work on Ribosome in Brookhaven National Laboratories till 1995. Finally, in 1999 he joined as a Professor at the University of Utah in Biochemistry Department. Lastly, he moved to his current position as a Senior Scientist and Group Leader in Structural Biology studies at MRC, Laboratory of Molecular Biology, Cambridge, UK, from where he received this noble prize.
Dr. Thomas A. Steitz was born in 1940 in Milwaukee, Wisconsin, USA. He received Ph.D. from Harvard Universit y in 1966 in Biochemistry and Molecular Biology. Since1970, he was associated with Yale University as a Faculty and also as an Investigator at Howard Hughes Medical Institute. Thomas Steitz is a co-founder and chairperson of Scientific Ad visory Board of a company, Rib-X pharmaceutical, involved in the translation studies of the ribosome. It could be applied also for the development of effective antibiotics against micro-organisms resistant to drugs.

Thomas Steitz's scientific career has involved in studies of biological structure using X-ray crystallography including developing and refining of novel techniques to determine the structure of proteins and nucleic acids. Dr. Steitz has mostly emphasized on understanding the structural basis of enzymes and protein-nucleic acid interactions. His structural studies on the large ribosomal subunit provided the first atomic level insights into the structure and function of the ribosome. He solved the famous phase problem in crystallography which was the major barrier of structural studies of ribosome . The high resolution structure of 50S subunit of Ribosome at 2.4Å published by Thomas A. Steitz and his collaborators in 2000 is marked as the milestone in Ribosome Studies.[3]

Dr. Ada Yonath was born in Jerusalem, Israel. She earned her B.Sc. (1962) and M.. Sc. (1964) degrees from Hebrew University. In 1968, she completed her Ph.D. from the Weizmann Institute (WI) of Science, Israel in X-Ray Crystallography. In her postdoctoral work in USA, at Carnegie Mellon University and at the Massachusetts Institute of Technology (MIT), she continued her studies on X-Ray Crystallography. Later in the early 1970s, she initiated her famous research on determination of X-ray crystallographic structure of Ribosome. In 1980, for the first time she showed the well ordered 3D microcrystal of 50S ribosomal subunits from *Geobacillus stearothermophilus* in *in vitro* condition.[18] Later, she changed her focus of research on 30S ribosomal subunit. In the year 2000, she published an article on structure of functionally activated small ribosomal subunit at resolution of 3.3Å along with her co-workers[13]. Since then, she is recognized as a pioneer researcher in the field of Ribosomal Crystallography.

Currently, she is holding the position of chief scientist of the two research groups at Max Plank Research Unit, Hamburg, Germany and Mazer Centre of Structural Biology, WI, Israel. Dr. Yonath was recently elected to be the member of the Israeli Academy for Science and Humanities, a prestigious society that is akin to the US National Academy of Sciences. She was also the first winner of the New European Crystallography Prize, which was established last year to recognize significant achievements in the past 5 to 10 years for European crystallographers.

Elucidation of their Nobel Winning Work

It is an uphill task to crystallize ribosome due to its enormous size (2.5 MDa) and thoroughly irrelevant shape. Ribosome is the chief workshop of protein synthesis. Crystal structures of the ribosome opened a new gateway in understanding the protein synthesis machinery. Initial work was started by Dr. Ada Yonath and other collaborators in 1980 at the Weitzman Institute of Science in Israel. Later Dr. Thomas A. Steitz began his research in 1995 on larger ribosomal subunit along with his research group at Yale University, USA., whereas Dr. V. Ramakrishnan mostly focused on smaller subunit of Ribosome (30S) (Table1).

Table1:	Summary of important breakthrough in ribosomal crystallography in chronological order
1980	Dr. Yonath first reported the 50S subunit crystals of Ribosome from *G. stearothermophilus*[6]. Phase problem was solved by Dr. Steitz and collaborators.
1991	3Å resolution structure of 50S subunit from *H. marismartui* reported by Dr. Yonath and collaborators[8]
1999	Application of cryo-crystallography in determining Ribosome structures which minimized the radiation damage of ribosome crystals[9] 5Å resolution structure of 50S subunit (*H. marismartui*) was reported by Dr. Steitz[10] 5.5Å resolution of 30S subunit structure of Ribosome (*T. thermophilus*) was reported by Dr. Ramakrishnan[11] followed by Dr. Yonath's 30Sat 4.5Å. resolution[12].
2000	Dr. Steitz gave 2.4Å resolution structure of 50S subunit (*H. marismartui*)[5]. Dr. Ramakrishnan gave 3Å resolution structure of 30S subunit (*T. thermophilus*)[13] followed by Dr. Yonath's 3.2 Å structure with corrections at atomic level[14].
2001	Dr. Yonath gave 50S high resolution structure from *Deinococcus radiodurans* which was suitable for Antibiotic targeting of the Bacterial Ribosome[15]. Noller and collaborators gave 5.5Å resolution structure of 70S subunit from *T. thermophilus* [16] and then Cate and collaborators gave it at 3.5Å from *E. coli*[17].

Ramakrishnan's Contribution to Ribosome Research

Ramakrishnan's crystallographic work for 30S ribosome subunit helped in a great deal for the better understanding of the whole ribosome structure and its protein machinery, which is responsible for protein synthesis. Ramakrishnan in the year 2000 proposed 30S structure from *T. thermophilus*[17] diffracted at high resolution such as 3.0Å or less but it still needed some more refined crystallographic structures to understand the various processes such as tRNA and peptidyl transferase selection. Later in the year 2005 he proposed that the ribosome could be activated or deactivated by mutating at the ribosomal RNA and proteins.

Importantly, he found that some antibiotics have intense effect on the accuracy of codon reading. Till date it remained unexplainable that, how this remote controlled codon-anticodon interactions takes place in protein synthesis.

The elucidation of 30S structure revealed numerous important aspects related to translational event and importance of ribosomal subunit in this process. Entire ribosome has three tRNA sites such as the A (aminoacyl) site, the P (peptidyl) site and the E (exit) site. After mRNA binds to the so called Shine and Dalgarno (SD) leader sequence in 16s rRNA of 30S subunit, initiator tRNA charged with formylated methione binds to the P site in 30S subunit. After this initiation process 50 subunit docks to the A site of 30S subunit and elongation process starts. In this process, aminoacyl tRNA, EF-tu and GTP forms a complex molecule and move to the A site. EF-tu accommodates aminoacyl tRNA in the A site followed by GTP hydrolysis. Hence, Aminoacyl tRNA became peptidyl-tRNA after peptide bond formation, occupies still the A site, it is a pre-translocation state. In the next elongation step, another elongation factor G (EF-G, a GTPase) upon binding to the A-site, translocate the peptidyl-tRNA from A to the P site and move empty tRNA from A to the E site.

In this way, a site is being ready to accommodate a new aminoacyl tRNA according to the codon sequence of mRNA and polypeptide chain elongates with one amino acid. This polypeptide elongation continues until the stop codon appear in the A site. Hydrolysis of GTP to GDP plays an important role in this entire process. This is also suggested that, cognate tRNA more efficiently induced conformational changes in the ribosome such that, it changed to a productive form which accelerates GTPase activation. This entire process is summarized in the figure 1 given below.

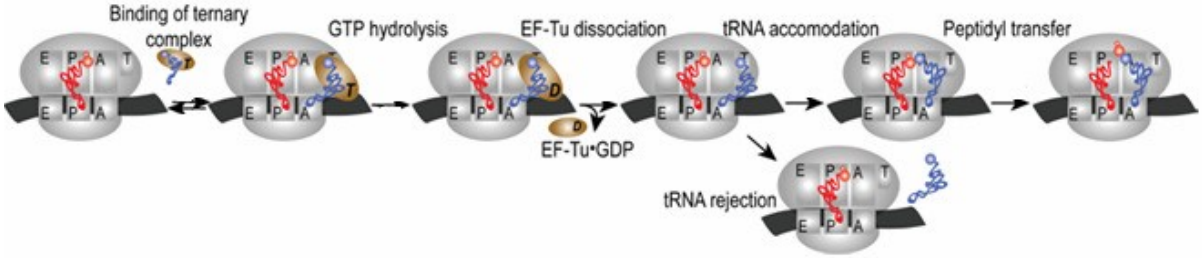

Figure 1: Schematic Representation ribosomal decoding and peptidyl-transfer (From Ogle and Ramakrishnan, 2005, reproduced with permission).

Recently, Dr. Ramakrishnan and his collaborators clearly explained some unanswered important question about the functionality of small subunit of the ribosome. We know so far that, 30S subunit starts the initiation of protein synthesis. In their recently published work they showed, 1) how the selection and proofreading process works between the initial tRNA and 30S subunit? 2) They also explained about the wobble mechanism; 3) How mutated ribosome and antibiotic controlling the accuracy of protein synthesis by ribosome?

He studied the effects of binding antibiotic Paromomycin to 30S subunit of Ribosome, which stabilized the conformation of two universally conserved bases A1492 and A1493 of internal loop of helix 44 of 16S rRNA. Also, it was found that binding of tRNA causes the change in the G530 base which is present as a pseudo knot at 16S RNA. This provides the structural explanation of the wobble hypothesis as, these three bases lines the codon-anticodon helixes in such a way that, the first two base positions are checked but not the wobble or 3rd one.

It is known that, after the initial process starts by 30S subunit, 50S subunit docks to the A site of the smaller subunit. Total subunit 70S, then go for the elongation and completion of protein synthesis. In ideal conditions, both the subunits attain a specific conformation in total 70S ribosomal unit to produce highly accurate polypeptide chain. Dr. Ramakrishnan group showed that, the accuracy of protein synthesis would depend on the position of two groups of protein in ribosome The open conformation (increased accuracy) is stabilized by ribosomal proteins S4 and S5, while the closed conformation (decreased accuracy) is stabilized by ribosomal protein S12. These groups of protein could be controlled either by mutation or by antibiotic treatment. Hence, their work is producing a new avenue for controlling the protein synthesis at this level[11]. The figure 2 below explains the opening and closing of ribosomal 30S subunit.

Ribosomal Subunit Structures and Antibiotic Discovery:

Ribosome is a crucial molecule for all living organisms including prokaryotic and eukaryotic systems, hence can be targeted for designing new drugs. Recent crystallographic structures of the ribosome have shown a new way for the development of the new superior antibiotics for all resistant strains of bacteria. Till date out of total antibacterial drugs, half of the drugs are developed by targeting the ribosomes only

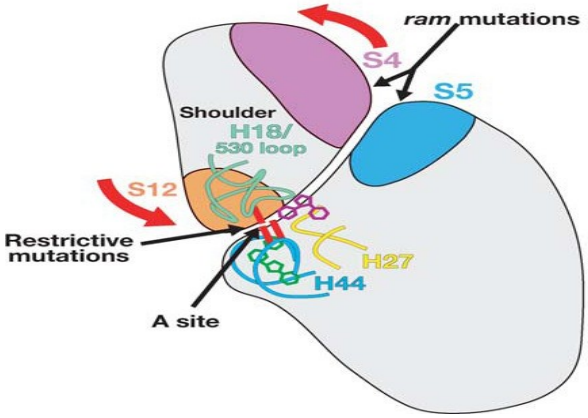

Figure 2: Closing and opening of the 30S subunit. The subunit opens and closes by intra-subunit rotations(red arrows) around the A site.(From Ogle and Ramakrishnan, 2005, reproduced with permission).

Table 2: Mode of action of various antibiotics on different ribosomal subunits

Ribosomal Subunit	Antibiotic class	Antibiotic	Mode of action
30S	Aminoglycosides	Paromomycin	Bind to A or P sites and affect codon- anticodon Interaction.
	Tetracycline	Streptomycin	
	Others	Geneticin	
		Tobramycin	Block binding of A-site to tRNA
		Tetracycline	
		Edeine	Inhibit Translocation
		Pactamycin	
		Spectinomycin	
50S	Macrolides	Azithromycin	Block peptide bond formation by interfering with A or P site tRNA and/or prevent elongation of nascent peptide
		Erythromycin	
		Carbomycin	
		Clarithromycin	
		Spiramycin	
		Roxithromycin	
		Troleandomycin	
	Ketolides	ABT-773	
		Telithromycin	
	Streptogramins	Dalfopristin	
		Quinupristin	
		Virginiamycin 5	
		Virginiamycin M	
	Lincosamides	Clindamycin	
	Pleuromutilins	Titamulin	
	Phenyl propanoids	Chloramphenicol	
	Oxazolidinones	Linezolid	
	Others	Puromycin	
		Anisomycin	
		Blastocidin	
		Sparsomycin	

Recent advancement in the crystallographic structure of ribosome, showing new avenues for the developments of novel drugs, against these newly emerging drug resistant pathogens Interaction studies have shown the way how these antibiotics could act on each ribosomal subunit. Table 2 shows how the antibiotic affects the different subunits of the ribosome. They are developed mostly from the strain *T. thermophilus and* the detailed structural information are available in a research article published by Franceschi and Duffy (2006)[6].

Nano biotechnology is producing a new horizon for early detection of a disease and subsequent drug delivery. This technology is allowing us to deliver drug at the individual cellular level, after detecting the disease at the rudimentary stage. These newer drugs targeting ribosome could be utilized to block the protein synthesis in each individual diseased cell as well. Thereby, toxicity to the healthy cells could be nullified. Hence, the combination of new drug and nanotechnology could revolutionize the field of medicine. Nano-biotechnological approaches are still in the proposed stage[7].

Conclusion

This year, Nobel committee felicitated three eminent scientists Ada E. Yonath, Thomas A. Steitz and Venkatraman Ramakrishnan due to their pioneering work on the protein factory of the cell. Pharmacological research will get new impetus all over the world due to their ground breaking work on ribosomal crystallography. We are hoping that, this result will help to us to find more specific antibiotics for this dreadful disease in the near future. All Indian are especially proud for Dr. V. Ramakrishnan due to his potential contribution in this field. However, we must not forget the contributions of other renowned scientist in this field, such as, Henry Noller at University of California, Santa-Cruz and Peter Moore at Yale University**.** In last January Dr Yadugiri published the latest interview of Dr Ramakrishnan where the readers will get Dr Ramakrishnan's view regarding Indian Science and future potentials (Yadugiri 2010) [21].

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