The Ribosome and the 2009 Nobel Prize in Chemistry

Laasya Samhita and Umesh Varshney


Laasya Samhita (left) is presently pursuing PhD in the Department of Microbiology and Cell Biology at the IISc, Bangalore. Her research interests include evolutionary biology, currently addressing evolutionary questions in protein synthesis.

Umesh Varshney (right) is a Professor in the Department of Microbiology and Cell Biology at the IISc, Bangalore. His research group uses *Escherichia coli* and mycobacteria as model organisms, and exploits various heterologous systems to address mechanistic issues relevant to protein synthesis, and DNA repair pathways using biochemical and molecular genetics approaches.

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The 2009 Nobel Prize in Chemistry was awarded jointly to Venkatraman Ramakrishnan, Thomas Steitz and Ada Yonath "for studies on the structure and function of the ribosome". The ribosome is a massive molecular machine comprising proteins and RNA and is responsible for the synthesis of proteins, often termed the workhorses of the cell. Proteins are key players in almost all intracellular transactions and hence the importance of the ribosome.

Information Transfer from DNA to Proteins

Although DNA, often referred to as the blueprint of life, has today become almost a catchword, proteins are somewhat less spoken of outside of academia, possibly because of their bewildering variety and varied structures. In fact, it was for this reason that in the late 1930's to mid 1940's, proteins were considered far stronger candidates to form the genetic material, than was DNA.

When Crick (together with Watson and Wilkins) received his Nobel Prize in Medicine or Physiology in 1962 for solving the structure of DNA, his Nobel lecture was dominated by the excitement of unravelling the genetic code and finding out how proteins are made. To quote him, "It now seems certain that the amino acid sequence of any protein is determined by the sequence of bases in some region of a particular nucleic acid molecule". The year 1962 also saw the Chemistry Nobel Prize being shared by Kendrew and Perutz for solving the three-dimensional structures of haemoglobin and myoglobin, by X-ray crystallography. X-ray crystallography as a technique to study molecular structures predates the excitement about molecular biology, and was developed by the father and son team of the Braggs for which also the Nobel Prize was awarded (in Physics) in 1915. The awards recognized the ingenuity and skill that went into deciphering the three-dimensional structure of a macromolecule using X-ray

crystallography, a technique that is still maximally used to solve protein structures.

The last Nobel Prize related to ribosome function, awarded jointly to Holley, Khorana and Nirenberg in 1968 in Physiology or Medicine for their "interpretation of the genetic code and its function in protein synthesis", was the most critical in understanding how DNA-based life functions. Their findings confirmed Crick's earlier hypothesis that DNA encodes instructions according to which amino acids are assembled and joined together to form proteins. In 1958, Crick had suggested the presence of an intermediate 'adapter' molecule attached to an amino acid which would direct it to its proper position next to a corresponding triplet code. The breakthrough came the same year with the discovery of transfer RNA (tRNA), first identified as a soluble RNA or 'sRNA'. Work on the genetic code led to the discovery of 64 possible triplet base combinations of which 61 could be translated into specific amino acids (the remaining three signalled the end of protein synthesis). However, organisms appeared to possess around half this number of distinct tRNA molecules. It followed from this that each tRNA molecule may recognize more than one triplet code to mean the same amino acid.

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Crick proposed a mechanism entitled 'the wobble hypothesis' to explain how this could happen. He postulated what was later found to be true, that of the three bases of a triplet code, the third base does not encode critical information. It is the first two that decide which tRNA (aminoacyl-tRNA) will be picked. For instance, UUU and UUC encode the amino acid phenylalanine and they can both be decoded by a single tRNA having GAA anticodon. The puzzle however remained, that clearly tRNA molecules are all very alike in their three-dimensional structure; how then does the ribosome tell them apart on the basis of just the two bases? Also, for example, as in the case of UAG (which encodes a stop codon) and UAU (which encodes tyrosine), it is necessary to discriminate even at the third base position. How this occurs at the molecular level has only recently been understood, thanks largely to the micro-view of the decoding centre offered by the

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Both crucial discoveries, that information in DNA encodes protein, and that there is a requirement of an intermediate to do so, were instances where the theory preceded the discovery. This emphasizes the importance of free thinking in science. In parallel with these discoveries, in the early 1950's, Paul Zamecnik and co-workers had already developed a cell-free system using rat liver to demonstrate protein synthesis. When radioactively labelled amino acids were added to the mixture, the radioactivity was found to disperse throughout the system over some time. However, when the process was stopped quickly, it was found that the radioactivity was concentrated in specific areas of the cell. These areas coincided with the nucleoprotein particles of ~10 μm diameter earlier recorded as dense particles in the electron microscopic images from the cytoplasm of various living cells. With the development of high speed centrifugation methods, these particles could be separated from other intracellular particles and studied, and with the discovery of tRNA, a cell-free assay could be set up to show that indeed these particles serve as the sites for protein synthesis. In 1958, at a meeting of the then new Biophysical Society, R B Roberts proposed the term 'ribosomes' for particles that contained complexes of one third to one half RNA and two thirds to one half protein, were ~10-15 μm in diameter, had sedimentation values in the range 20 to 100S, were found in all cell types and seemed 'somehow to be involved in protein synthesis'. The name proved popular and has remained to this day.

Elucidation of Ribosome Structure

Ribosomes are the protein factories of the cell.

Ribosomes are the protein factories of the cell. They exist in all cells in all life forms. In eubacteria, each ribosome can be divided into two sub-units of 30S and 50S, according to their sedimentation coefficients. Each ribosome is about 2.6 million Dalton in size. To understand how the ribosome works, it was necessary to pool data from biochemical experiments (which make use of the purified components *in vitro* to simulate the conditions for protein synthesis), genetic experiments (which exploit the impact of

various mutations and their suppressors in various factors to carry out *in vivo* studies), and structural analyses of the various factors at high resolution. This last aspect has only recently been achieved through advances in crystallisation.

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To crystallize an asymmetric and immensely complex molecule of this size was seemingly an impossible task. It was Ada Yonath, then at the Max Planck Institute in Berlin, who in the early 1970's first sought to make it possible. She decided to map the exact location of each atom in the ribosome using X-ray crystallography. For this she used the bacterium *Geobacillus stearothermophilus* (earlier *Bacillus stearothermophilus*) which is found in hot springs and can survive at temperatures up to 75°C. Her idea was that ribosomes from such an organism are likely to be relatively stable. By 1980, she had indeed managed to produce the first diffracting crystals of the large (50S) subunit of the ribosome. It took over twenty more years to obtain a high resolution structure of the ribosome. Ada Yonath pioneered the field and her spirit and innovative techniques inspired others to join the research in this challenging and exciting field.

Although Ada Yonath's structure could place most atoms in their relative positions, this did not help decipher the three-dimensional structure of the ribosome. Thomas Steitz at Yale took the problem forward by using images of the ribosome that were generated by cryoelectron microscopy as reference. Using this approach, it was possible to identify the location and orientation of specific regions within the ribosome crystals. These images, along with other conventional techniques culminated in translating the two-dimensional scatter of the X-rays by the ribosome crystals into a three-dimensional arrangement of atoms. By 1998, Steitz's group succeeded in solving the structure of the large subunit of the ribosome from a halophilic (salt loving) bacterium *Haloarcula marismortui* at ~9 Å resolution. Although by the standards of crystallography this was by no means a high resolution structure for the ribosome it was a major breakthrough; besides, one could visualize the strands of RNA molecules within the structure. Meanwhile the methods to isolate ribosomes,

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techniques to crystallize them, as well as methods of crystal structure analysis including computational capacity and expertise were progressing side by side.

Venkatraman Ramakrishnan from MRC Cambridge joined in the quest by starting work on crystallizing the small (30S) subunit of the ribosome. In the year 2000, the breakthrough arrived, with three groups independently deciphering the high resolution (~3Å) structure of the large or small ribosomal subunits. While Ramakrishnan and Yonath generated structures of the 30S subunit from the bacterium *Thermus thermophilus,* Steitz obtained better resolution crystals of the 50S subunit from *Haloarcula*. We should add that Harry Noller's laboratory at the University of California, Santa Cruz, also contributed a great deal in our understanding of the ribosome biology including solving of the three-dimensional structures of the ribosome at high resolutions.

What Does the Structure Tell Us?

With the ribosome structures in hand, one could now ask far more specific questions with regard to the functioning of the ribosome than was possible before. For instance, it would be clear from their relative locations, which RNA nucleotides are placed near which proteins within the ribosome, and therefore how they might influence the primary function of the ribosome, that of decoding the genetic code and building polypeptide. Also, it served to confirm or reject earlier evidence gathered from genetic and biochemical data that had served to build a model of how the ribosome functions. In turn, it also offered clever ways to design genetic and biochemical experiments, emphasizing the diverse points of investigation needed to understand a biological problem in its entirety.

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To understand what the ribosome structure has offered, it is necessary to know how it drives protein synthesis. Each ribosome has three distinct sites for binding tRNAs, the 'A' (aminoacyl) site, the 'P' (peptidyl) site and the 'E' (exit) site. These sites stretch across both the 30S and the 50S subunits (*Figure* 1). The

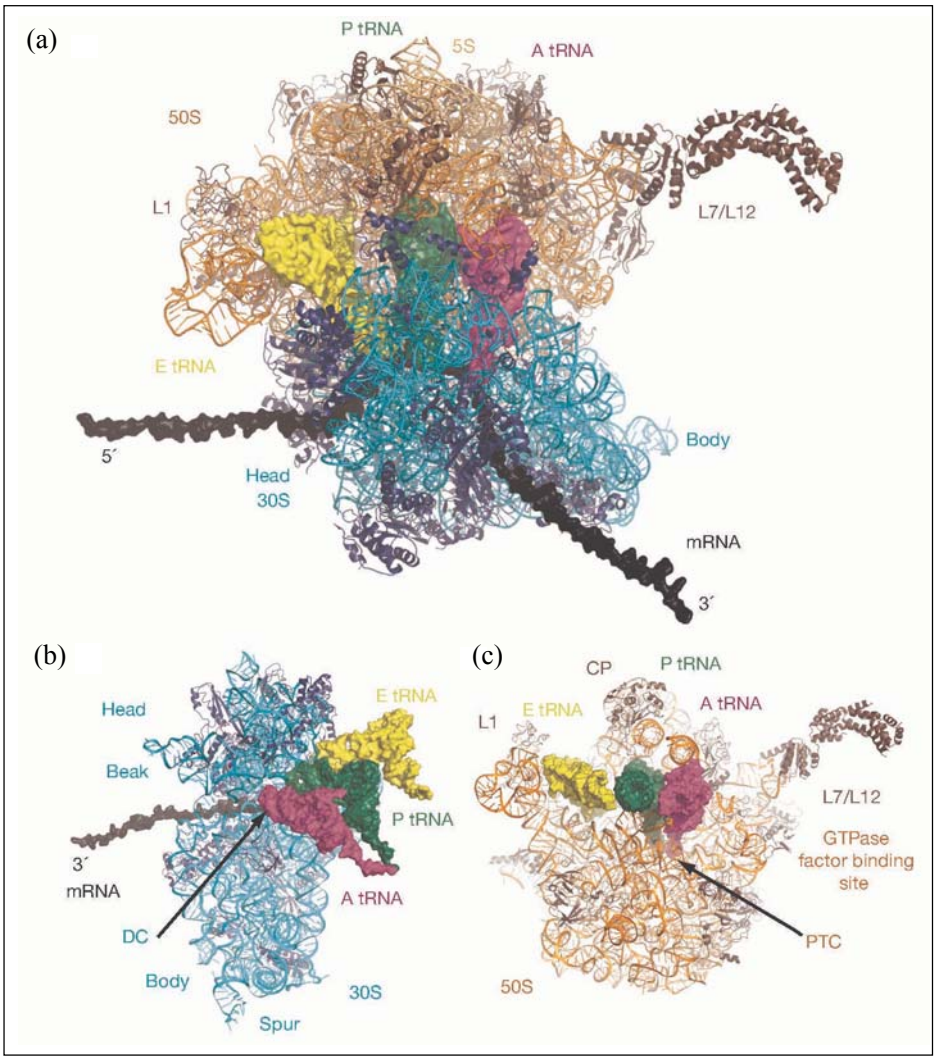

Figure 1. Overview of the bacterial ribosome: (a) A view of the bacterial 70S ribosome from the top, showing the A-site, P-site and E-site bound tRNAs in purple, green and yellow, respectively. (b) and (c) Split views of the 30S and 50S subunits. Locations of the anticodon and acceptor end regions of the bound tRNAs in 30S and 50S subunits, respectively. The decoding centre (DC) in the 30S subunit and the peptidyltransferase centre (PTC) in the 50S subunit are marked with arrows. Various other regions of the 30S and 50S subunits are as shown. EF-Tu and EF-G binding to the ribosome is facilitated by the L7/L12 proteins in the ribosome (a, c). The mRNA (elongated by modeling) is shown in black (a) or in grey (b). The interface between the two ribosomal subunits consists largely of RNA.

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process of translating an mRNA molecule into a polypeptide or protein can be broken down into four main steps, initiation, elongation, termination and ribosome recycling. At the first step, the mRNA binds the 30S subunit of the ribosome, followed by binding of the initiator tRNA attached with formylmethionine, to the P-site. This reaction is aided by three initiation factors, IF1, IF2 and IF3. Once the initiator tRNA and the mRNA are aligned properly, the 50S subunit 'docks' onto this complex, thus assembling a functional 70S ribosome. In the next step, the elongation step, the empty A-site receives an elongator tRNA carrying the second amino acid of the protein to be synthesized. The A-site receives an aminoacylated tRNA bound to elongation factor-Tu (EF-Tu) in complex with GTP. If this incoming tRNA has an anticodon that is complementary to the mRNA codon in the A-site, GTP hydrolysis occurs and is followed by peptide bond formation with the amino acid attached to the tRNA in the P-site. Peptide bond formation is catalyzed in the peptidyltransferase centre located in the 50S subunit. The ribosome now has a peptidyl-tRNA in the A-site, and a deacylated tRNA in the P-site and is referred to as being in a 'pre-translocation' state. With the aid of elongation factor- G (EF-G, also bound to GTP), the peptidyl-tRNA in the A-site is 'translocated' to the neighbouring P-site while the P-site tRNA (deacylated) is moved to the E-site from where it exits the ribosome later. This is referred to as the 'post-translocation' state of the ribosome.

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A large body of biochemical work by Harry Noller and his group led to the realization of a 'hybrid state' proposal. Accordingly, prior to the binding of EF-G to the ribosome, the tRNA in the A-site moves to the P-site on the 50S subunit, while remaining in the A-site of the 30S subunit (the 'A/P' state). Similarly the P-site tRNA moves to the E-site on the 50S subunit while remaining in the P-site of the 30S subunit (the 'P/E' state). The function of EF-G then translocates them to the classical P- (or

'P/P') and the E- (or 'E/E') sites, respectively. While these movements occur in the ribosome, the tRNAs remain stably bound to the cognate codons on the mRNA to ensure maintenance of the reading frame. The repeating cycles of elongation (increase of the peptide chain by one amino acid) and translocation (movement of the tRNAs and the mRNA in the ribosome) continue until a stop codon appears in the A-site. Stop codons are recognized by release factors, RF1 or RF2 in bacteria, which aid in detaching the polypeptide by hydrolysing the ester bond that connects its C-terminal end with the 3' OH group in the P-site bound tRNA signalling the 'termination' step of protein synthesis. Subsequently, in the ribosome recycling step, the two ribosomal subunits as well as the deacylated tRNA are disassembled to free them for newer rounds of translation by a protein called the ribosome recycling factor (RRF) along with EF-G. Furthermore, there exists another special release factor called RF3 (a GTP binding protein) which facilitates the recycling of RF1 and RF2. As is clear from these details, the process of protein synthesis has evolved to accommodate several factors. The relative concentrations of these factors within the cell are regulated to orchestrate successive rounds of translation with high fidelity.

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The decoding centre (on the 30S subunit, marked DC in *Figure* 1b) where the genetic code in the mRNA is read to pick a specific tRNA, and the catalytic centre (on the 50S subunit marked PTC in *Figure* 1c) where successive amino acids are joined to form a polypeptide, are the two fundamental active centres of the ribosome. In the decoding centre, the ribosome performs the task of matching the codon in the mRNA with the anticodon in the tRNA that carries a specific amino acid. In this process, the ribosome has to both select the right tRNA and discard all the wrong ones that may attempt to pair with a given codon. All tRNAs are alike in their three-dimensional structure, and for their recruitment in the A-site, they all bind to a common factor, EF-Tu which guides them to the ribosome. To select a tRNA on the basis of just two discriminatory bases in the anticodon region, and to discard others that do not have these two bases is a daunting task. To add

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to this, the process has to be not only highly accurate but also highly efficient. Although earlier kinetic and biochemical studies had shown that the ribosome rejects the wrong tRNAs with a high degree of precision, it was not known by what means the ribosome performed this monitoring. The insight came with Ramakrishnan's crystal structure of the 30S subunit complexed with an mRNA and a cognate tRNA at the A site of the ribosome. Once the tRNA binds in the A-site, three of the residues (G530, A1492 and A 1493) of the ribosomal RNA in the 30S subunit in the decoding centre, change their conformations to 'inspect' the codon-anticodon base pairing by sensing the geometry of the minor groove that forms upon their pairing (*Figures* 2a,b). In the process, the first two base pairs of the codon-anticodon pair establish interactions within the decoding centre (*Figures* 2c–2e). The nature of these interactions is such that only the correct base pairing (as established by the standard Watson–Crick pair-ing rules) is stabilized, allowing for further steps to occur. Incorrect base pairings will lead to insufficient stabilization of the loosely bound tRNA, which then falls off the ribosome. Also, it was revealed that the entire 30S subunit undergoes a conformational change to a 'closed' form upon binding of the correct tRNA in the A-site to further facilitate the retention of the selected tRNA.

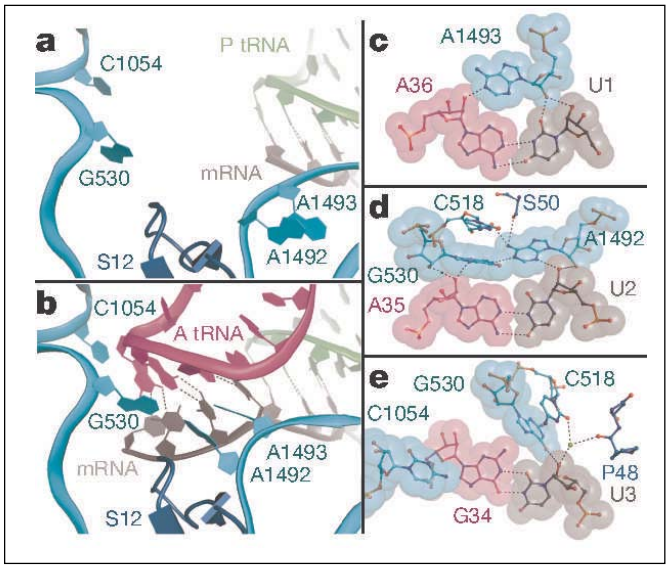

Figure 2. Decoding by the ribosome (a) shows the positions of ribosomal RNA residues (critical in decoding) in the decoding centre prior to the entry of the tRNA into the A site (b) shows the change in conformation of these residues upon pairing of the codon (5'-UUU-3' in grey) and anticodon (5'-GAA-3' in purple). (c) and (d) show how the ribosomal RNA bases A1493; and A1492 and G530 scrutinize the geometry of pairing of the first two bases of the codon (U1 and U2) with the corresponding bases in the anticodon. (e) Nature of the ribosomal RNA interactions with the third position codon-anticodon pair shows that it can be flexible. Interactions of the ribosomal protein S12 with the second and the third positions in the codon (U2 and U3) are also shown.

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The second functional centre of the ribosome is the peptidyl transferase centre. This is where the peptide bond formation occurs to link the amino acids together to form a polypeptide. In addition, the hydrolysis of peptidyl-tRNA (during the termination step) also occurs here. The first high resolution image of the catalytic site was provided by the 50S structure solved by Steitz and colleagues in 2000. The structure showed that there were no proteins within ~18 Å radius of the catalytic site, indicating that the ribosome functioned as a ribozyme, an RNA enzyme (although we now know that ribosomal proteins also contribute to enhancing this activity). Evidence from the structures suggests that the tRNAs in the A- and the P- sites are held in very precise positions in the peptidyl transferase centre in the 50S subunit. So the ribosome appears to promote the reaction by favourable alignment of the substrate (*Figure* 3). This reduces the free

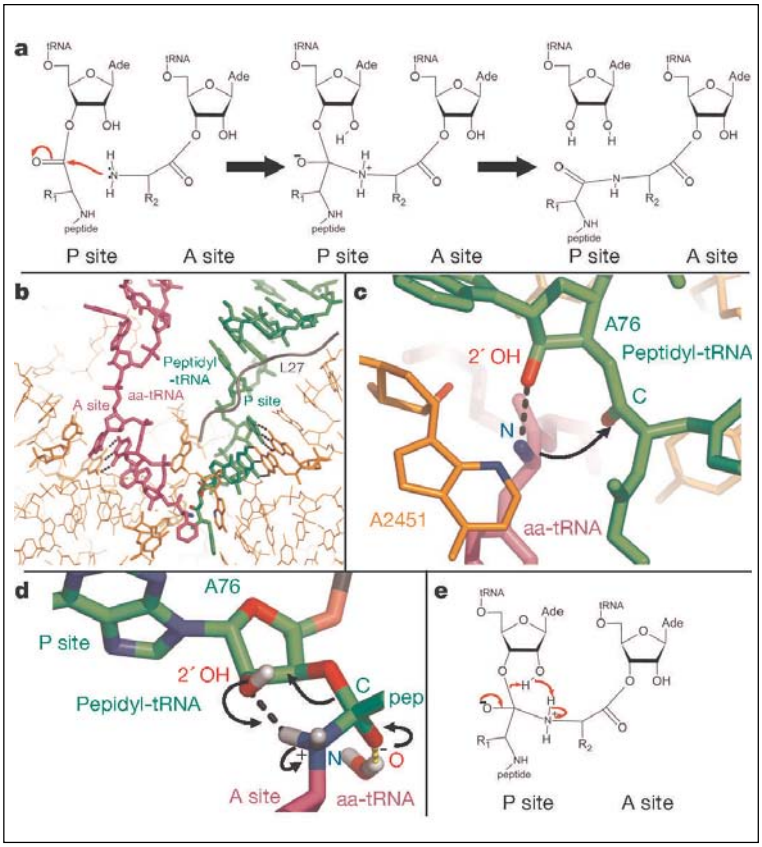

Figure 3. Peptide bond formation

(a) ***Schematic of the chemical reaction of peptide bond formation.*** **(b)** ***Binding of the aminoacyl-, and peptidyl-, tRNAs (shown in purple and green, respectively) in the peptidyl-transferase centre (PTC).*** **(c)** ***Positioning of the α-amino group of the aminoacyl-tRNA (in the A-site) is aided by the 2' OH of the 3' terminal residue (A76) of the peptidyl-tRNA in the P-site and the ribosomal RNA residue (A2451), to launch a nucleophilic attack on the ester bond carbon atom of the peptidyl-tRNA to form a peptide bond.*** **(d) and (e)** ***Depict a possible mechanism by which the reaction intermediates break down to yield the products.***

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energy required to form a peptide bond. Recent structures of ribosome with the release factors have also shed light on the second function, that of termination, carried out by the peptidyl transferase centre. The release factors (RF1 and RF2 in bacteria) recognise the stop codons with high specificity and trigger hydrolysis of the ester bond between the polypeptide and the tRNA in the P-site to release the polypeptide chain.

Future Implications

Aside from the mechanistic insights, visualizing the bacterial ribosome in atomic details also meant that one could now design new ways of targeting the various activities it performs to stop protein synthesis. Bacterial ribosomes are distinct from those of humans and many of the antibiotics in use today work by interfering with bacterial protein synthesis. In fact, several antibiotics have been co-crystallized with ribosomes, showing exactly where they bind and how they block or ruin the process of accurate protein synthesis. In recent years, resistance against antibiotics has become a major health concern with pathogenic bacteria evolving mutations in the ribosomal RNA or in ribosomal proteins to prevent inhibition of the ribosome by the antibiotic. Knowing the structure of the ribosome and the nature of the acquired mutations in the ribosomal components helps us to redesign the antibiotics. In addition, work on the structure of the ribosome has had a tremendous impact on experiments and experimental designs that seek to gain a better understanding of ribosome biology. Also, much of biotechnology dealing with engineering higher yields of proteins of medicinal and other commercial importance would benefit quite directly from ribosome research. A better understanding of how the ribosome works therefore assumes high socio-economic importance. Already work is underway to exploit the information that the crystal structures have unveiled. The next few years will see more experimentation and a detailed study of the available structures of the ribosome as well as those that will be solved to capture the snap shots of the ribosome in various phases of its action. We have come a long way from the time when it was first discovered

Evidence from the structures suggests that the tRNAs in the A- and the P- sites are held in very precise positions in the peptidyl transferase centre in the 50S subunit.


Venkatraman Ramakrishnan


Thomas Steitz


Ada Yonath

that almost all of life is DNA based and functions on a near universal genetic code. The ribosome remains the most awe-inspiring perhaps of all intracellular structures, continuing to throw up challenges and surprises.

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Address for Correspondence
Laasya Samhita
Umesh Varshney*
Department of Microbiology
and Cell Biology
Indian Institute of Science
Bangalore 560 012, India.
Email:
aysaal_ls@yahoo.co.in
* uvarshney@gmail.com