Nobel Prize in Chemistry

DOI: 10.1002/anie.200905795

Nobel Prize for the Elucidation of Ribosome Structure and Insight into the Translation Mechanism**

*Knud H. Nierhaus**

electron microscopy · ribosomes · X-ray diffraction · structure elucidation · structural biology

In 1999, four papers reported crystal structures of ribosomal particles at modest resolution (ca. 5 Å),[1–4] indicating that we stood on the brink of a scientific breakthrough, and in particular in the field of protein synthesis. In fact, the revolution in structure resolution was dramatically improved within the following few years: In 2000 and 2001, a second wave of papers from the same groups were published that reported ribosomal subunits at high resolution (2.4–3.0 Å)[5–7] and the complete ribosome at 5.5 Å.[8] This work sent shockwaves through the ribosome community: After about a decade of slow progress, we suddenly had the structure of one of the most complicated complexes of cell, the ribosome, directly in front of our eyes. The ribosome transforms the genetic information encoded within the genes into proteins formed from strings of amino acids, and has been present since the beginning of the development of the first cells on earth about 3.5 billion years ago. It was immediately clear that a breakthrough of this magnitude should be honored with a Nobel Prize, but the dilemma immediately became obvious: Four groups were involved, but a Nobel Prize can be shared only by three.

For those who worked near or in the field of translation and ribosome structure, this revolution did not fall from the sky. Ada Yonath (Figure 1) followed an invitation of Heinz-Günther Wittmann, director of the Max Planck Institute for Molecular Genetics in Berlin, in the late seventies to study the ribosome structure using X-ray crystallography—in those days a brave task, considered to be almost impossible. Wittmann developed his department in the Berlin institute in the seventies and eighties into a Mecca for ribosome research. In the late eighties, he and Masayasu Nomura were considered to be prime candidates for the Nobel Prize. In Nomura's laboratory, the procedure for reconstitution of the small ribosomal subunit from its components was developed, which indicated that the ribosomal components contained the necessary information to adopt the correct ribosome structure ("the wonder of Masayasu"). Nomura founded our understanding of translational control of the synthesis of ribosomal proteins in Madison, and continued in Irvine with groundbreaking research concerning ribosome biogenesis in eukaryotes and the organization of the nucleoli.[9]

Figure 1. The three Nobel laureates Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath (Scanpix/AFP/US; Michael Marsland/Yale University; Micheline Pelletier/Corbis).

It was Yonath and Wittmann who first demonstrated that it was indeed possible to crystallize ribosomal particles, the first crystals being those of the large subunit isolated from the extremophile *Bacillus stearothermophilus*, which was published in 1980.[10] Nevertheless, it still took 15 years until good and well-diffracting crystals were achieved, and significant technological improvements paved the way to collecting satisfying diffraction patterns, five years after the premature death of Wittmann in 1990. Such improvements included Yonath's introduction of cryogenic conditions for the ribosome crystals, without which the crystals would be fried by the enormous luminescence of the synchrotron beam before diffraction data could be collected. Other important technical developments included improving the intensity of the synchrontron beams themselves and improvements in the sensitivity of the detectors. The Wittmann crystallography group in Berlin was taken over by Francois Franceschi (now at Rib-X in New Haven, USA), and it was under his supervision that practically all the crystals were prepared. The data were collected and processed by Ada Yonath and her co-workers, who moved to the Max Planck Outstation at DESY in Hamburg.

Thomas Steitz (Figure 1) had in his entourage two excellent young scientists, Nenad Ban and Poul Nissen, both of whom have continued their outstanding work in Zürich and Aarhus, respectively. I remember Tom mentioning, after the youngsters left his group having significantly contributed to

[*] K. H. Nierhaus
Max-Planck-Institut für Molekulare Genetik, AG Ribosomen
Ihnestrasse 73, 14195 Berlin (Germany)
E-mail: nierhaus@molgen.mpg.de

[**] I thank my colleagues Markus Pech, Christian M. T. Spahn, and Daniel N. Wilson for help and discussions.

the definition of ribosome crystallography in the Steitz group, that he could hardly imagine having such excellent co-workers ever again in his life (but than, he added, it happened again). The strength and excellence of the Steitz group is due to his strong dedication to enzymes and factors of the molecular genetics (replication, transcription, and translation), with the result that the plethora of structures that his group has solved in the last decades has enjoyed widest attention.

Venki Ramakrishnan (Figure 1) is the youngest of the three laureates, and I have to say he is one of the most ingenious colleagues I have ever met. This is illustrated by the fact that during his career he changed topics within the large ribosome-research field several times: first starting with Don Engelman and Peter Moore performing neutron-scattering analysis of the small ribosomal subunit, then entering the crystallography of ribosomal proteins, and then suddenly (for us it seems almost from nothing) to become one of the leading figures in ribosome crystallography. Venki combines a comprehensive knowledge of ribosomal functions with that of technical demands of structure research, loves and knows classical music (his son plays the cello in a young and gifted quartet), speaks Spanish, and reads papers in Russian.

Before we have a look to the specific achievements of the laureates and the revolutionary importance of their work, let us briefly go back in time to around 1990, when the first promising diffraction patterns were obtained but the phasing of which was however not yet possible. Regarding the crystallization technique, the input of a Russian group in Pushchino[11] has to be mentioned, who published a crystallization procedure for *Thermus thermophilus* ribosomes just before the Yonath group; the ribosomes of this bacterial strain developed into a model ribosome for ribosome crystallography. One member of the Russian group was Marat Yusupov, who ten years later, together with Jamie Cate, were the crystallization promotors in Harry Noller's group. And again, these young scientists also continued their outstanding work on ribosomes as independent researchers in Strasbourg and Berkeley, respectively.

Around 1995, the nasty obstacle of the phase problem called for fresh ideas. Three different strategies were applied that finally opened the window to high-resolution structures; two of these strategies were used in a paper by the Steitz group in 1998:[12] 1) cryoelectron microscopic (Cryo-EM) structures of the large ribosomal subunit from Joachim Frank in Albany (now in Columbia, New York) helped during the first steps, and phases were computed according to the classical technique of molecular replacement; 2) isomorphous replacement with heavy-atom clusters led to experimental phases, as shown previously with nucleosomes;[13] and 3) a third strategy was the exploitation of the strong anomalous scattering of some heavy metals used previously for the structure determination of smaller enzymes.[14]

What then are the essential outcomes of the revolution in structural biology? We summarize some outstanding features:

1. Protein synthesis happens at the interface between both subunits in the 70S ribosome, which is practically free of proteins (Figure 2A,B). The two essential centers of the ribosome, the decoding center on the small subunit and the peptidyltransferase center on the large subunit, are mainly composed of rRNA, which prompted Tom Cech to make the famous statement "*The ribosome is a ribozyme*".[15]
2. A ribosome contains 50 to 70 proteins, depending on the strain/organism. Despite the large number, the rRNAs are usually the dominating components, with two-thirds of the ribosomal mass in bacteria (half in eukaryotes and one-third in some mitochondria). Many ribosomal proteins exhibit the peculiar feature of having a globular domain, which is usually present at the ribosome surface, with long extensions protruding into the ribosome (Figure 2C) that play important roles for assembly and stability of this particle.
3. The advent of the crystal structures instantly increased the known RNA structures tenfold. Despite this enormous progress, it was astonishing that only two new motifs for RNA structures were observed, namely the A-minor motif and the K-turn. The A-minor motif (see Figure 2D) is characterized by an adenine nucleotide that folds into the minor groove of a helix, thus making H-bonds with the 2′-OH groups of a single base pair.[16] 186 of these adenines have been identified in the large ribosomal subunit of *H. marismortui* and are therefore one of the most prominent elements to maintain the stability of the three-dimensional structure of ribosomes. Furthermore, A-minor motifs play an important role at the heart of the ribosomal decoding process (see below) and at the peptidyltransferase center. The second motif, the K-turn, is of minor importance; it connects two helices that form an angle of 120° owing to a kink of the phosphodiester chain within an internal loop between the two helices.
4. All three laureates recognized from the very beginning that ligands, such as antibiotics, could be soaked into ribosomal crystals, thus enabling the specific binding site of the drug to be precisely characterized. This effect is not only important in understanding the mechanism of action of antibiotics and their resistance mechanisms, but also identifies regions of the drugs that can modified to improve binding with the aim to increase its medical efficiency—all aspects that are of great interest for the pharmaceutical industry. Owing to the many possible points of interference, the ribosome is one of the major targets in the cell for antibiotics. It is clear that the ribosome structures will play a central role for future drug design to develop better inhibitors.
5. One of the landmarks of the Ramakrishnan work was the deciphering of the principles behind the decoding mechanism at the A-site of the small ribosomal subunit. For decades we assumed that the ribosome measures the stability of base pairing of the codon–anticodon interaction at the decoding center in the A-site to ensure fidelity, the consequence would be that AU-rich codon–anticodon interactions should be more error prone than GC-rich. However, Siv Andersson and Chuck Kurland published a report 25 years ago[17] that confused us by showing that this was not true, because both kind of codons were decoded with the same precision. The structures determined by Ramakrishnan solved this riddle:[18] As an example, the

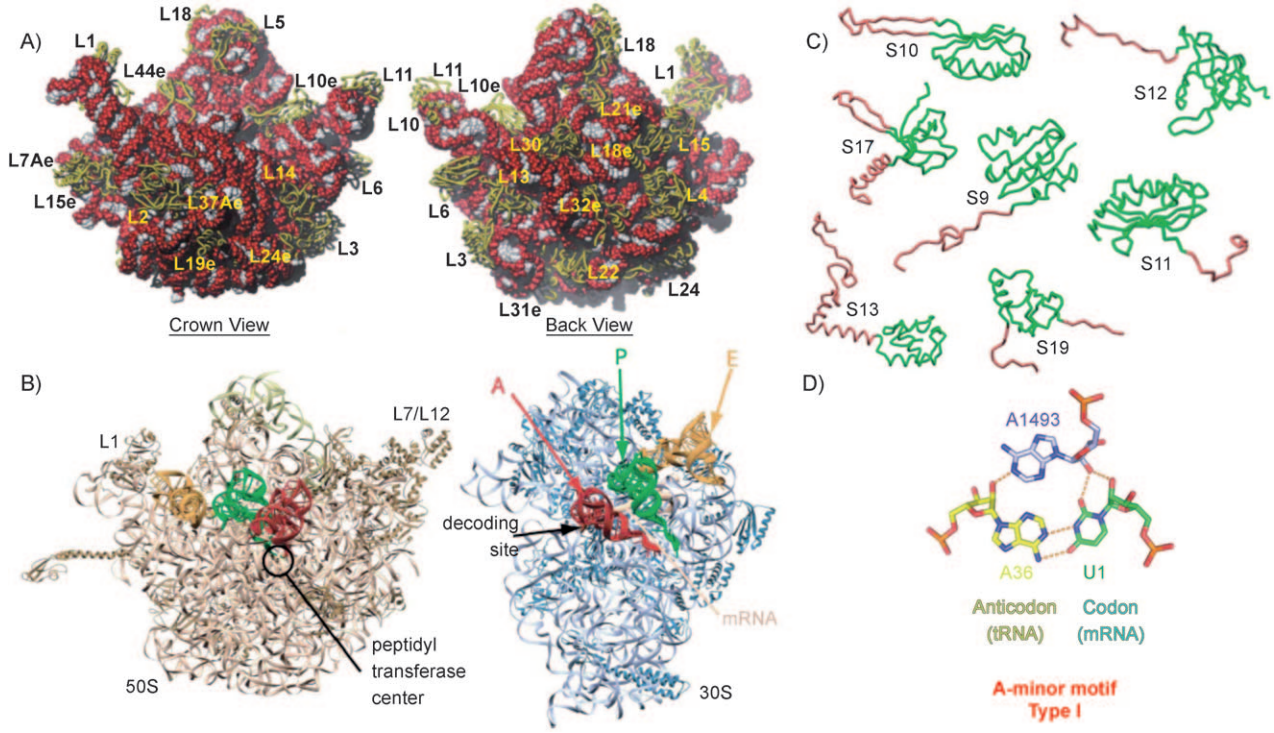

Figure 2. Landmarks of the ribosome structure. A) Two views of the large ribosomal subunit from the archaea *H. marismortui*: left: the interface side, which shows no ribosomal proteins (yellow) in the middle, where the peptidyltransferase center is located; right: the cytoplasmic side, where the ribosomal proteins are evenly distributed over the surface.[22] B) The interface side of both subunits, with the attachment sites of a tRNA, when it passes through the three ribosomal binding sites A, P, and E during protein synthesis. Brown: large subunit, blue: small subunit.[23] C) Examples of the structure of some ribosomal proteins of the small subunit.[24] D) The first base-pair of the codon–anticodon interaction in the decoding center. For explanations, see the text.[18]

first base-pair of the codon–anticodon interaction is shown in Figure 2D with the U1 residue from the *U*UU codon and the residue A36 from the 3′-nucleotide of the anticodon. The universally conserved A1493 residue flips into the minor groove of the base-pair and forms three sequence-independent hydrogen bonds, two with the 2′-OH groups of both ribose residues and one with the O2 residue of the uracil. The latter hydrogen bond is also unspecific: if a purine is at this position of the codon, an N3 residue would occupy the same position and like O2 would also act as a proton acceptor. Only in the case of a correct (cognate) Watson–Crick interaction would the H-bonds have an optimal geometry and thus the highest bond energy with the highest stability. An incorrect base-pair (near-cognate codon–anticodon interaction) would have less stability; the energy difference is the discrimination energy for selection of the correct aminoacyl tRNA. The important consequence of this mechanism is that it is not the stability of the base-pair but rather the correctness of the spatial geometry of a Watson–Crick pair that is measured by the decoding center. The same principle holds true for the middle base-pair of the codon–anticodon duplex, with even more discrimination energy involved, whereas the third base-pair does not follow this rule and does not strongly contribute to the discrimination in the wobble position. In fact, a mistake in decoding because of a mistake in the third position usually has no dramatic consequences for folding, structure, and function of a protein (see Ref. [19] for a discussion).

6. After the publication of the Steitz group, the structure of the large ribosomal subunit triggered a heated discussion about the peptide bond mechanism of the ribosomal peptidyltransferase center and a flood of ingenious mutagenesis studies from various laboratories to test the involvement of conserved nucleotides at the center. The result, together with a molecular dynamic study, was a satisfying picture of the molecular mechanism of the only enzymatic activity of the ribosome.[20]

As mentioned at the beginning, the reason for the delay in awarding the Nobel Prize for ribosome structure was the fact that too many excellent groups had to be considered. In addition to the three laureates, at least two more were considered to be prime candidates for the Prize: Harry Noller (Santa Cruz) and Joachim Frank (Albany). The Noller group published the first 70S ribosome structures carrying mRNA and tRNAs at all three tRNA binding sites A, P, and E (Figure 2B), thus providing valuable clues for the binding of the tRNAs and bridges between subunits.[8] One year before, Joachim Frank's group determined the structure of the most important elongation-cycle complexes using samples prepared in my group, which was the basis for the first video describing protein synthesis derived from experimental results.[21] The power of cryo-EM is seen by the fact that

about 300000 particles can be used for a highly-resolved ribosome reconstruction, with a resolution of down to 5.5 Å, which then can be used to fit the known atomic structures of the ribosome, yielding a pseudo-atomic resolution. 300000 particles are an extremely small amount of ribosomes; note that one pmol contains 6×10^{12} particles! Many of the functional complexes have not yet yielded crystals, but most of them yield cryo-EM reconstructions, provided that a homogenous population is present in the sample. Multi-particle cryo-EM can even give us insight into structural dynamics of the ribosome in solution.

The previous paragraph already implies that the crystal structure of the ribosome is not the end of ribosome research, but rather the opposite is true. The crystal structure allows the design of tailored biochemical experiments, and with the advent of new techniques, such as single-molecule measurements, the final goal of ribosome research, namely to understand the functions and dynamics of the ribosome, comes within reach. The molecular details of tRNAs moving through the ribosome, beginning from A-site occupation, moving via two translocations to P and E sites, from which it is released, are far from being understood. Consider that a tRNA is a large molecule of the size of an average cytoplasmic protein, and two of these large molecules together with and connected to the mRNA by codon–anticodon interactions have to move through the ribosome in a precise fashion. Finally, the structure of a eukaryotic ribosome is not known; it would be desirable to have a structure of a higher eukaryote such as rat, rabbit, pig, or man. A comparison with the structure of the bacterial ribosome would be of utmost importance for designers of pharmaceuticals.

The decision of the Nobel Committee was extremely difficult. Figure 3 shows the Committee during the announcement of the prizewinners; on the right side sits Måns Ehrenberg, a distinguished scientist in the ribosome field from Uppsala, who has made important contributions concerning function and (together with the Frank group) structure of functional complexes. He no doubt played a decisive role in the wise decision of the Committee. We have to congratulate the laureates for their outstanding achievements, and we also applaud the Committee.

Figure 3. Announcement of the winners of the Nobel Prize in Chemistry 2009 (dpa).

Received: October 15, 2009
Published online: November 7, 2009

[1] A. Tocilj, F. Schlünzen, D. Janell, M. Glühmann, H. Hansen, J. Harms, A. Bashan, H. Bartels, I. Agmon, F. Franceshi, A. Yonath, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14252.
[2] W. M. J. Clemons, J. L. May, B. T. Wimberly, J. P. McCutcheon, M. S. Capel, V. Ramakrishnan, *Nature* **1999**, *400*, 833.
[3] N. Ban, P. Nissen, J. Hansen, M. Capel, P. B. Moore, T. A. Steitz, *Nature* **1999**, *400*, 841.
[4] J. H. Cate, M. M. Yusupov, G. Z. Yusupova, T. N. Earnest, H. F. Noller, *Science* **1999**, *285*, 2095.
[5] N. Ban, P. Nissen, J. Hansen, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 905.
[6] F. Schluenzen, A. Tocilj, R. Zarivach, J. Harms, M. Gluehmann, D. Janell, A. Bashan, H. Bartels, I. Agmon, F. Franceschi, A. Yonath, *Cell* **2000**, *102*, 615.
[7] B. T. Wimberly, D. E. Brodersen, W. M. Clemons, R. J. Morgan-Warren, A. P. Carter, C. Vonrhein, T. Hartsch, V. Ramakrishnan, *Nature* **2000**, *407*, 327.
[8] M. M. Yusupov, G. Z. Yusupova, A. Baucom, K. Lieberman, T. N. Earnest, J. H. Cate, H. F. Noller, *Science* **2001**, *292*, 883.
[9] M. Nomura, *J. Biol. Chem.* **2009**, *284*, 9625.
[10] A. Yonath, J. Müssig, B. Tesche, S. Lorenz, V. A. Erdmann, H. G. Wittmann, *Biochem. Int.* **1980**, *1*, 428.
[11] S. D. Trakhanov, M. M. Yusupov, S. C. Agalarov, M. B. Garber, S. N. Ryazantsev, S. V. Tischenko, V. A. Shirokov, *FEBS Lett.* **1987**, *220*, 319.
[12] N. Ban, B. Freeborn, P. Nissen, P. Penczek, R. A. Grassucci, R. A. Sweet, J. Frank, P. B. Moore, T. A. Steitz, *Cell* **1998**, *93*, 1105.
[13] T. V. O'Halloran, S. J. Lippard, T. J. Richmond, A. Klug, *J. Mol. Biol.* **1987**, *194*, 705.
[14] W. I. Weis, R. Kahn, R. Fourme, K. Drickamer, W. A. Hendrickson, *Science* **1991**, *254*, 1608.
[15] T. Cech, *Science* **2000**, *289*, 878.
[16] P. Nissen, J. A. Ippolito, N. Ban, P. B. Moore, T. A. Steitz, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4899.
[17] S. G. Andersson, R. H. Buckingham, C. G. Kurland, *EMBO J.* **1984**, *3*, 91.
[18] J. M. Ogle, F. V. Murphy, M. J. Tarry, V. Ramakrishnan, *Cell* **2002**, *111*, 721.
[19] V. Di Giacco, V. Marquez, Y. Qin, M. Pech, F. J. Triana-Alonso, D. N. Wilson, K. H. Nierhaus, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 10715.
[20] M. Pech, K. H. Nierhaus, *Chem. Biol.* **2008**, *15*, 417.
[21] R. K. Agrawal, C. M. T. Spahn, P. Penczek, R. A. Grassucci, K. H. Nierhaus, J. Frank, *J. Cell Biol.* **2000**, *150*, 447.
[22] D. Klein, P. Moore, T. Steitz, *J. Mol. Biol.* **2004**, *340*, 141.
[23] V. Ramakrishnan, *Cell* **2002**, *108*, 557.
[24] V. Ramakrishnan, P. B. Moore, *Curr. Opin. Struct. Biol.* **2001**, *11*, 144.

