CONTRIBUTIONS to SCIENCE, **7** (2): 125–130 (2011)
Institut d'Estudis Catalans, Barcelona
DOI: 10.2436/20.7010.01.117 ISSN: 1575-6343
www.cat-science.cat

The race to resolve the atomic structures of the ribosome. On the Nobel Prize in Chemistry awarded to Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath*

Bárbara M. Calisto, Ignacio Fita

Molecular Biology Institute of Barcelona-Spanish National Research Council (IBMB-CSIC), Barcelona Science Park, Barcelona

Resum. El Premi Nobel de Química 2009 va ser atorgat a tres científics, Venkatraman Ramakrishnan, Thomas A. Steitz, i a Ada E. Yonath, per les investigacions sobre l'estructura i el funcionament dels ribosomes. Els ribosomes són les partícules cel·lulars complexes en què es produeix la descodificació de la informació genètica i la síntesi de proteïnes i, conseqüentment, tenen un paper central en la biologia de tots els éssers vius. Tots els ribosomes es componen de dues subunitats, la petita i la gran, que en procariotes s'especifiquen com la subunitat 30S i la 50S, respectivament, segons les seves propietats de sedimentació. Aproximadament, dos terços de la massa de cada subunitat correspon a RNA ribosòmic (rRNA) i la resta, a proteïnes diferents. Atesa la rellevància biològica que tenen, i el fet que siguin dianes d'una gran varietat d'antibiòtics d'ús clínic, els ribosomes han estat l'objectiu d'una recerca intensa i contínua des dels anys seixanta, quan va ser desxifrat el codi genètic. Aquestes investigacions han conduït, i en cert sentit culminat, als resultats publicats l'any 2000 (*annus mirabilis* per al món del ribosoma) sobre la determinació de les estructures cristal·lines de la subunitat ribosomal 50S de *Haloarcula marismortui* a una resolució de 2.4 Å i, unes setmanes més tard, de la subunitat 30S de *Thermus thermophilus* a una resolució de 3.3 i 3.0 Å, pels equips que dirigien els premiats amb el Premi Nobel de Química 2009. Aquests resultats estan essent instrumentals per a la comprensió, atòmicament, del funcionament del ribosoma. Tanmateix, encara es necessiten anys de treball per a contestar les moltes preguntes obertes que romanen sobre els ribosomes, entre les quals cal ressaltar les referents a l'estructura del ribosoma eucariòtic.

Paraules clau: ribosoma · cristal·lografia de raigs X · síntesi proteica · codi genètic

Abstract. The Nobel Prize in Chemistry 2009 was awarded to three scientists, Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath, for their investigations into the structure and functioning of ribosomes. These complex cellular particles are where genetic information is decoded and proteins are synthesized. Consequently, ribosomes play a central role in the biology of all living organisms. Ribosomes are composed of one small and one large subunit, which in prokaryotes are respectively referred to as 30S and 50S according to their sedimentation properties. In both subunits, about two thirds of the mass corresponds to ribosomal RNA (rRNA) and the rest to different proteins. Given their biological relevance and the fact that they are the target of a large variety of clinically relevant antibiotics, ribosomes have been the subject of intense and continuous research since the 1960s, when the genetic code was unraveled. These investigations led, and to some extent culminated, with the results published in 2000 (*annus mirabilis* for ribosomes), reporting the crystal structures of the 50S ribosomal subunit from *Haloarcula marismortui* at 2.4Å resolution and, a few weeks later, of the 30S subunit from *Thermus thermophilus* at 3.3Å and 3.0Å resolution, by teams led by the three laureates. These results have been instrumental in understanding ribosome function at the atomic level. However, there are many years of work ahead, as much remains to be learned about ribosomes; in particular the structure of the eukaryotic ribosome has yet to be elucidated.

Keywords: ribosome · X-ray crystallography · protein synthesis · genetic code

* Based on the lecture given by I. Fita at the Institute for Catalan Studies, Barcelona, on 16 December 2009, and at the Octubre Centre of Contemporary Culture, Valencia, on 13 January 2010 for the Nobel Prize Cycle. A previous version of this article was published by the authors in An Quím (2009) 105:286-289.

Correspondence: I. Fita, Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Parc Científic de Barcelona, Baldiri Reixac 4-8, Torre R, 3era Planta, E-08028 Barcelona, Catalonia, EU. Tel. +34-934034668. Fax +34-934020893. Email: ifrcri@ibmb.csic.es

The Swedish Royal Academy of Sciences distinguished the work of Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath (Fig. 1) by awarding these scientists the 2009 Nobel Prize in Chemistry, for their investigations on the structure and functioning of the ribosome. In particular, the three laureates led research teams that, working for the most part independently from one another, succeeded in determining at atomic resolution the structures of the two subunits that form the ribosome. The work of all three groups mainly relied on data obtained from X-ray crystallography studies.

Fig. 1. From left to right, Venkatraman Ramakrishnan, Thomas A. Steitz, Ada E. Yonath. © The Nobel Foundation. Photos: Ulla Montan.

Ribosomes are complex cellular particles that are the site of protein synthesis, which accounts for their central role in the biology of all living organisms. This process of protein synthesis is called *translation* because the genetic information encoded in the sequence of nucleic acids by the four letters of the nucleotide alphabet is converted into the language of proteins, expressed in the twenty letters of the amino-acid alphabet (Fig. 2). Ribosomes catalyze the formation of peptide bonds between amino acids. They also participate in the decoding of the genetic information by contributing the molecular machinery required for the specific recognition of messenger RNA (mRNA) and transfer RNA (tRNA) molecules, which underlies the fidelity of the translation process. All ribosomes consist of two subunits, one small and one large. In prokaryotes, they are referred to, respectively, as 30S and 50S (Fig. 3) according to their sedimentation properties. Ribosomal RNA (rRNA) accounts for roughly two thirds of the weight of each subunit, while the remainder is made up of proteins. The architecture and essential aspects of ribosome function have been conserved throughout evolution. Eukaryotic ribosomes differ from their prokaryotic counterparts in that they are larger and contain more components, consistent with the higher complexity of the processes that have to be performed, including regulatory functions and interactions with cellular membranes.

Ribosomes have three binding sites for tRNA molecules, designated *A* (for acceptor- or aminoacyl-), *P* (peptidyl-) and *E* (exit-). The three binding sites are located on both ribosomal subunits, with the *aminoacyl stem regions* of the tRNAs (Fig. 2B) binding to the large subunit while the corresponding *anticodon stem loops* bind to the small subunit. The small subunit is also responsible for binding to the mRNA molecule (Fig. 2). The two subunits act in concert during the subsequent process of *translocation*, involving the concerted movement and replace-

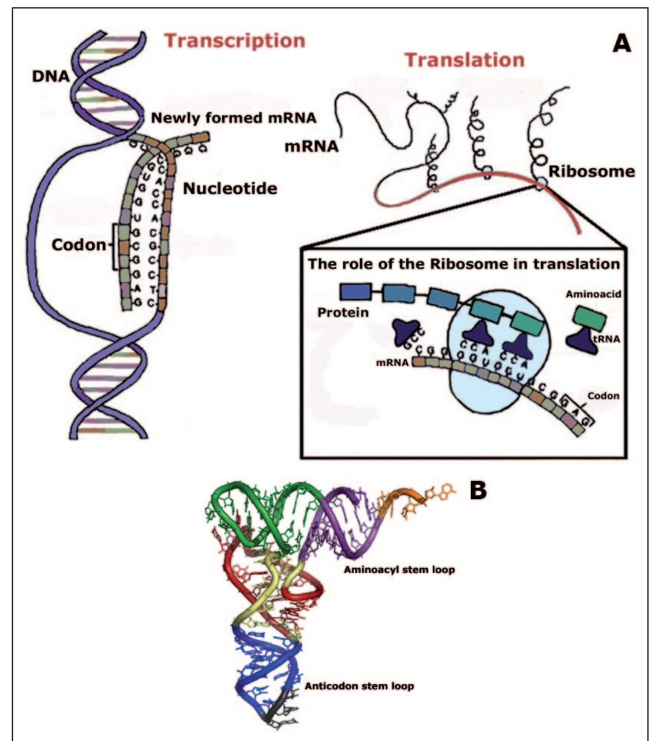

Fig. 2. The flow of genetic information and the role of ribosomes. (**A**) Genetic information in the form of DNA is converted into proteins in a process that involves two major steps. In transcription, the sequence of DNA nucleotides is transcribed into mRNA. During translation, mRNA is decoded to yield amino acids for the synthesis of proteins. (**B**) Structure of a tRNA molecule. The anticodon stem (blue) contains the three contiguous nucleotides of the anticodon (gray), which confer specificity to the tRNA. The aminoacyl stem (purple) contains the CCA 3′ tail (orange) where, according to the specificity of the tRNA, the corresponding amino acid binds.

PROKARYOTE (*E.coli*)

rRNA (nucleotides)	Protein	Subunits	Ribosome
23S RNA (3000), 5S RNA (120)	31 Proteins	50S (1.59×10^6D)	70S (2.52×10^6D); 21 nm; 29 nm
16S RNA (1500)	21 Proteins	30S (0.93×10^6D)	

EUKARYOTE (Rat)

rRNA (nucleotides)	Protein	Subunits	Ribosome
28S RNA (5000), 5.8S RNA (160), 5S RNA (120)	49 Proteins	60S (2.82×10^6D)	80S (4.22×10^6D); 22 nm; 32 nm
18S RNA (2000)	33 Proteins	40S (1.4×10^6D)	

Fig. 3. Comparative overview of the components of prokaryotic and eukaryotic ribosomes.

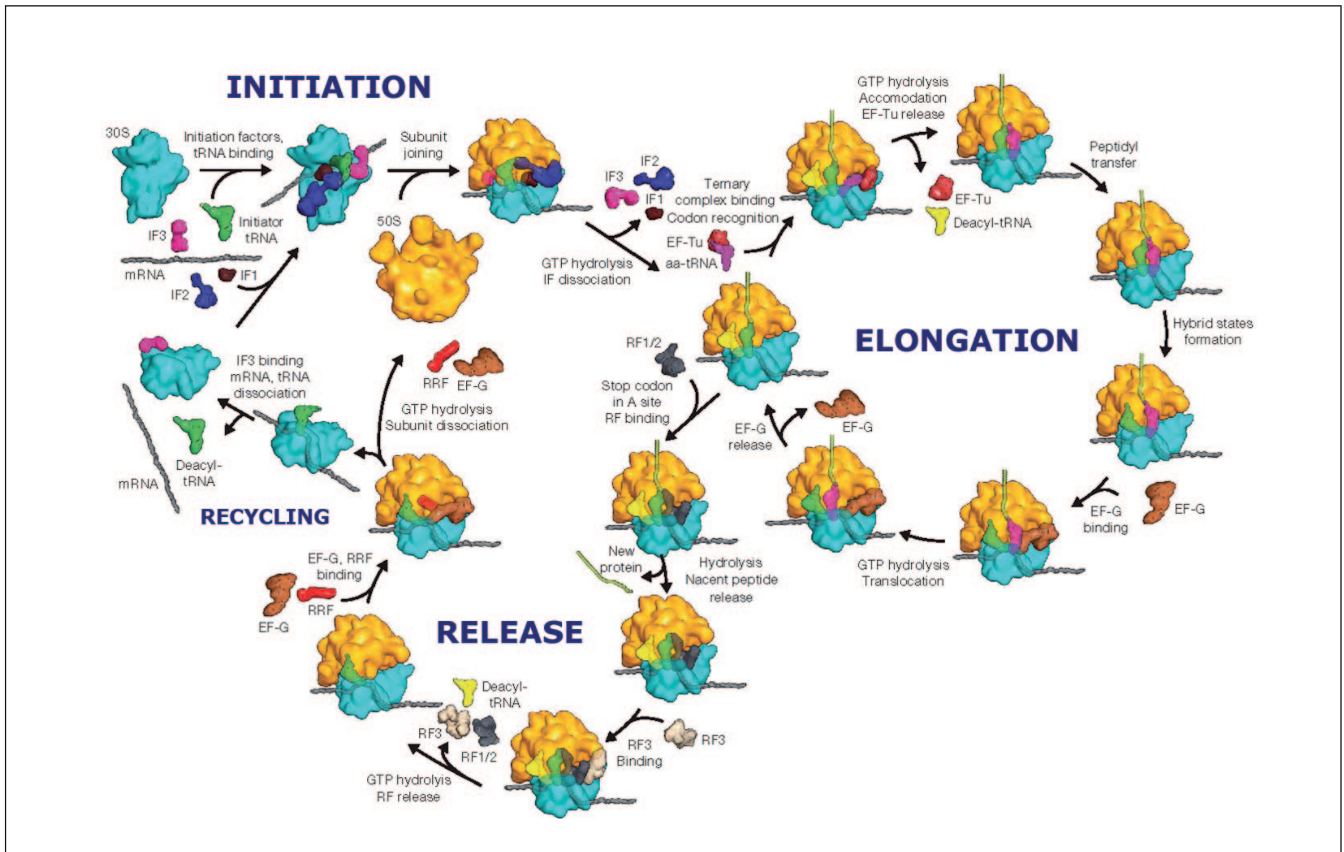

Fig. 4. 'Normal' working cycles in the prokaryotic ribosome. (Adapted directly from [10]).

ment of the three tRNAs molecules by precisely three nucleotides (comprising one *codon*) while the mRNA molecule moves forward with respect to the ribosome each time an amino acid is added to the nascent protein. The entire cycle requires energy, which is provided by the hydrolysis of several molecules of guanidine triphosphate (GTP), as well as several protein factors that interact transiently with the ribosome (Fig. 4).

Due to its biological relevance, the ribosome has been exploited as the target of a wide spectrum of clinically relevant antibiotics. The development of these drugs represents the intense and continuous investigations into the functional aspects of ribosomes using all available biochemical and biophysical techniques, based on the unraveling of the genetic code in the 1960s. These efforts led, and in many ways culminated, in the year 2000—the *annus mirabilis* for the ribosome world—with the determination of the crystal structures of the 50S subunit from *Haloarcula marismortui* at 2.4Å resolution [2] by Steitz and coworkers (Fig. 5A) and, just a few weeks later, of the 30S subunit from *Thermus thermophilus*, published independently at 3.3Å and 3.0Å resolution [9,15] (Fig. 5B) by the groups headed by the laureates Yonath and Ramakrishnan.

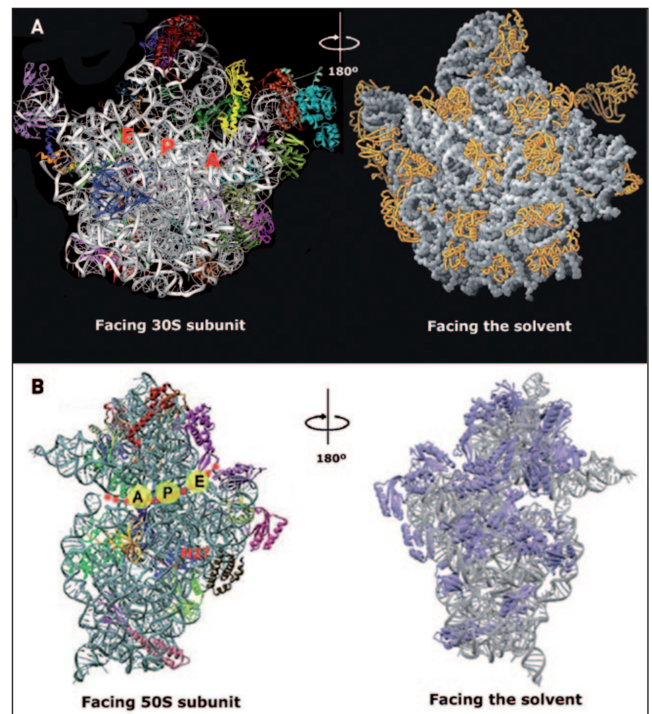

Fig. 5. (**A**)Structure of the 50S ribosomal subunit. On the right, the structure of ribosomal RNA (shown in gray) from *Haloarcula marismortui*, as reported in [2], and the associated proteins (shown in yellow). A-, P-, and E-tRNA binding sites are indicated on the left. (**B**) Structure of the 30S ribosomal subunit from *Thermus thermophilus*. On the right, the structure of ribosomal RNA (shown in gray) and the associated proteins (light purple) (adapted from [15]). A-, P-, and E-tRNA binding sites are indicated on the left.

The race to obtain the high-resolution structures

Solving the structures of the large and small subunits of the prokaryotic ribosome was accomplished using X-ray crystallography, as cited in the communication of the Swedish Royal

Fig. 6. Crystals of the 30S, 50S, and 70S ribosomal subunits.

Academy of Sciences. This technique provides structural information at the atomic or quasi-atomic level (i.e., resolutions greater than 3.5Å). However, there are two essential prerequisites for its success: the availability of suitable crystals (Fig. 6) and the ability to solve the *phase problem* for the diffractions obtained from the crystals. Given the size and complexity of the ribosomal subunits, it took decades to overcome these two requirements. The intense competition between several research groups no doubt resulted in important methodological progress and, eventually, scientific success.

Yonath, Wittmann and colleagues published the first crystals of the large ribosomal subunit (50S), from *Bacillus stearothermophilus,* in 1980 [16]. Five years later, the same group obtained, after numerous trials, better and more stable crystals of the 50S subunit, this time from a microorganism found in the extremely salty environments of the Dead Sea: *Haloarcula marismortui* [13]. Crystals optimized from these starting conditions finally allowed Steitz's group to determine the structure of the 50S subunit at atomic resolution, in August 2000 [2]. Surprisingly, although it was research from Yonath's group—especially the use of *H. marismortui* as a suitable source of the ribosomal samples—that produced the methodology needed to obtain crystals of the 50S subunit, Yonath is not a co-author of the papers reporting its three-dimensional structure.

The first crystals of the 30S ribosomal subunits, from *Thermus thermohilus*, were produced in 1987 in the USSR, by Sergei Trakhanov, Marat Yusupov, and colleagues [14]. However, later that same year, Yonath published similar crystals from the same thermophilic organism [6]. Those preliminary crystals, af-

Fig. 7. Structure of the whole 70S ribosome particle from *Thermus thermophilus*. The rRNA of the 30S ribosomal subunit is shown in light blue, the associated proteins in dark blue, the rRNA of the 50S ribosomal subunit in gray, and the associated proteins in purple. Three tRNA molecules occupying the A-, P-, and E-tRNA binding sites are shown in a color range from red to yellow (adapted from [17]).

ter many improvements, allowed the laureates Yonath and Ramakrishnan to solve the structure of the 30S ribosomal subunit at atomic resolution [9,15]. Crystals from the complete ribosome (the 70S particles), formed by the interaction of the 30S and 50S subunits, were also presented in the two resulting articles. Harry Noller (USA), in collaboration with Yusupov, solved the structures of the 70S particle and its complexes with tRNA and mRNA at about 5Å resolution (Fig. 7) between 1999 and 2001 [3,17]. It is difficult to avoid the polemics with respect to the importance and merits of the different findings and to the contributions of the scientists involved. It seems paradoxical that Yonath and Ramakrishnan independently and almost simultaneously solved the 30S subunit while working from the same microorganism, *T. thermophilus*. But perhaps the greater mystery is that elucidation of the complete ribosomal particle (70S), also from *T. thermophilus*, with a much higher complexity than the individual subunits and with important biological and functional implications, was not included in the work distinguished by the 2009 Nobel Prize in Chemistry. A possible explanation is that the resolution for the complete particle could not be considered as truly (quasi-) atomic. If it is indeed the case that the difference in resolution between 3.5Å and 5Å is critical to obtaining interpretable structures at the atomic level, then one could say that Noller and Yusupov were less than 2Å away from becoming Nobel laureates.

In 1998, Frank, Moore, and Steitz obtained the first convincing evidence that the phase problem could be solved for crystals from the ribosomal subunits. An electron-density map at 9Å resolution was obtained for crystals of the *H. marismortui* 50S ribosomal subunit in which fragments of the right-handed rRNA double helices could be recognized [1]. The decisive step in this work was the cryo-electron microscopy information provided by the laboratory of Dr. Frank, a co-author of the publication. Until then, electron microscopy had been the technique used to obtain the most important findings and contributions to the structural study of ribosomes, mainly due to methodological advances and the results of Drs. Frank (USA) and van Heel (Europe). Indeed, structural information at high resolution for particles as large as ribosomes can be obtained only with electron microscopy and X-ray crystallography. Electron microscopy has two main advantages over X-ray crystallography, both related to the above-mentioned requirements of the latter: in electron microscopy, crystals are not needed and the phase problem is solved experimentally by using electromagnetic lenses. However, electron microscopy also has a very important practical limitation, which is that only in the most favorable cases is it possible to attain quasi-atomic resolution; in studies of the ribosome particle, the best resolution has been about 6Å. Thus, despite its very important contributions, i.e., the versatility in sample selection and the speed of data analysis, electron microscopy has ceded ground to X-ray crystallography, also because structural information at atomic resolution is possible only with the latter technique.

To solve the phase problem in crystals of the ribosomal subunits, extended versions of classical protein crystallography methods were employed, including isomorphous replacement and anomalous diffraction. Improvements also followed from the use of compounds made up of clusters of heavy atoms, mainly tantalum and tungsten, such as $Ta_6Br_{12}^{2+}$ or ($K_6[P_2W_{18}O_{62}]\cdot nH_2O$), and by taking advantage of the strong anomalous diffraction from lanthanide ions and other elements, such as osmium and iridium [4]. The three laureates, especially Ramakrishnan and his group, contributed to these crucial technical advances.

Nonetheless, ribosome crystallography also presented a series of problems at the level of data collection and computation, neither of which could be addressed with the methodology available in 1980. However, in the mid-1990s, technological advances in bi-dimensional detectors, the availability of much more powerful computers, and the development of graphic systems, and elaborate crystallographic programs had opened the door to allowing structures as complex as ribosome particles to be solved. Other important methodological advances, in part propelled by the needs of ribosome crystallography, were the development of synchrotron light sources with adjustable wavelengths and cryo-crystallography, in which Yonath was a pioneer.

The ribosome at high resolution

The number of RNA structures known at the atomic level multiplied by a factor of 10 when the structures of ribosome were published in 2000. A similar breakthrough occurred regarding protein-RNA complexes. The huge amount of information obtained based on the structures solved by the three laureates has had important conceptual and practical implications. Here we limit our discussion to just a few aspects, concerning the biochemistry and structure of ribosomes.

The ribosome active site, where the peptide bond is formed, is the peptidyl transferase center (PTC). It is located in a region highly conserved in all ribosomes and it is exclusively formed by ribosomal RNA. Consequently, at the PTC, the ribosome acts as a ribozyme: an RNA enzyme. This mechanism presents a biological paradox in that proteins, responsible for essentially all enzymatic reactions that take place in living beings, depend on RNA as a catalyst for their own synthesis. The universality of the organization of the PTC in ribosomes, notably including the conserved presence of an internal two-fold symmetry, implies the existence of this catalytic center independently of environmental conditions . Accordingly, the PTC may well correspond to the remains of a primitive synthetic machinery, a proto-ribosome, capable of producing non-coded oligopeptides. This ancient organization recalls the existence of an ‘RNA-world’ during the initial stages of the evolution of life.

Since 2000, the three laureates, and many other investigators, have continued to use X-ray crystallography to investigate the structures of several ribosomal complexes and ribosomal variants [5,7,11,12]; but despite the enormous amount of information derived from those structures, now interpreted at atomic resolution, many of the details of ribosome functioning are still unknown. During protein synthesis, the ribosome performs very complex processes, including its displacement along the mRNA molecule and its use of amino acids that arrive with their corresponding tRNAs to build up the nascent polypeptide chain (Fig.

4). Besides this regular activity of protein synthesis, the ribosome is essential to error correction, interactions with regulatory factors and newly synthesized proteins, and the control of protein folding. However, thus far, the lack of a complete view of the functioning of the ribosome in all those processes, including the corresponding conformations and interactions, can be compared to seeking to make a movie but forced, at least for the time being, to accept a limited number of snapshots, in this case provided by the crystal structures. A persisting, if not impossible challenge is the difficulty in obtaining crystals suitable for high-resolution X-ray diffraction studies and representing many of the states through which the ribosome passes. At the same time, electron microscopy has gained renewed interest due to its enormous experimental possibilities and the use of the atomic information that is now available, It has therefore allowed significant advances to be made in achieving an integrated view of ribosome functioning [5,8,10]. Currently, the combination of X-ray crystallography and electron microscopy seems to be the most fruitful approach to the study of large macromolecular complexes such as the ribosome.

Conclusions

The results achieved by the three Nobel laureates were decisive in understanding ribosome functioning at the atomic level. Still, the answers to many of the remaining questions will require years of work. One of the most challenging problems is the high-resolution structural determination of the eukaryotic ribosome. The importance and singularity of the contributions of Ramakrishnan, Steitz, and Yonath, and the broad implications of their results, are beyond doubt. However, we must not forget the many other researchers whose investigations created the necessary framework. Their findings, in their originality and importance, are in some cases (Frank, Noller, Yusupov, Moore) as valuable as those of the 2009 laureates in Chemistry. It should also be noted that Ada Yonath is only the fourth woman, and the second who was not a Curie, to win the Nobel Prize in Chemistry. The previous female laureate in Chemistry, Dorothy Hodgkin, was awarded the Prize in 1964 “for her determinations by X-ray techniques of the structures of important biochemical substances.” A considerable amount of time has elapsed between the two Nobel Prizes in Chemistry awarded to women but, interestingly, both women were recognized for their research on major biological structures, accomplished using X-ray crystallography. The awarding of Nobel Prizes to X-ray crystallographers clearly reflects the fundamental knowledge gained by elucidating the atomic structure of complex molecular systems in order to achieve an in-depth understanding of their function.

The Nobel Prize in Chemistry 2009 awarded to Ramakrishnan, Steitz, and Yonath for “their studies on the structure and function of the ribosome” extends the already long list of Nobel Prizes in Chemistry that have distinguished research on biological systems. Overall, these prizes serve to remind us that biology, with its versatility, complexity, and possibilities, is what we would today refer to as ‘extreme chemistry’ and as such will continue to be a source of inspiration.

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