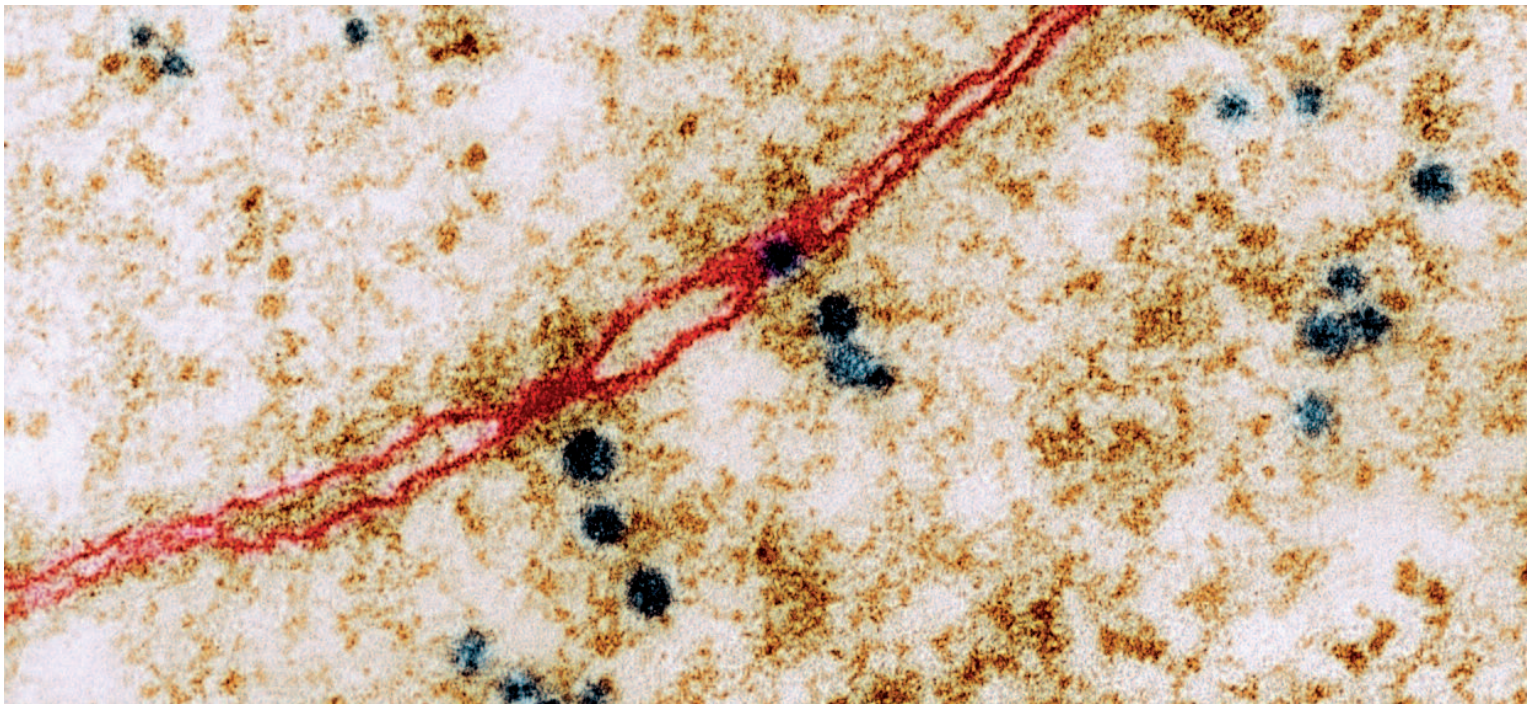


Coloured transmission electron micrograph of ribosomes (blue) passing through pores in a cell's nuclear membrane (red).
Photo: CNRI/Science Photo Library

The chemical basis for life
The 2009 Nobel Prize in Chemistry

PETER KARUSO

The story of the 2009 Nobel Prize in Chemistry is an epic in time and proportion. The Nobel committee has awarded the ultimate Prize in Chemistry for the structure and mechanistic interpretation of the ribosome.

At the beginning of the 20th century, the chemical foundations of life were a mystery. In the ensuing 100 years, these secrets have been slowly unlocked. This year's Nobel Prize completes a quadrella of prizes that celebrate molecular biology's central dogma: that DNA is transcribed to RNA, which is in turn translated to DNA (Crick 1970).

The first Prize, in 1962, was given to Watson, Crick and Wilkins for their discovery of the structure of DNA. The second was in 1969 to Arthur Kronberg and Svero Ochoa for their discovery of how DNA is copied and passed from cell to cell. The third was to Roger Kronberg in 2006, who discovered how cells transcribe DNA to mRNA. This year, to complete the journey from genome to proteome, the Nobel Committee has honoured Venkatraman Ramakrishnan (MRC Laboratory of Molecular Biology, Cambridge), Thomas A. Steitz (Yale) and Ada Yonath (Weizmann Institute of Science) '...for studies of the structure and function of the ribosome' or how simple mRNA is translated to complex (and functional) proteins. (http://nobelprize.org/nobel_prizes/chemistry/laureates/2009/press.html)

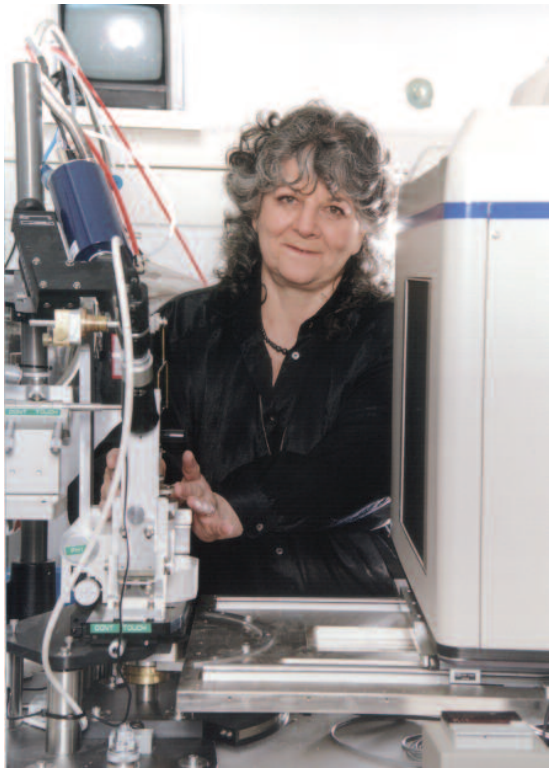
Inside every cell in every organism, there are DNA molecules that contain the blueprints for how that organism looks and functions. The blueprints become transformed into living matter through the work of ribosomes – huge complex machines which synthesise every protein based on a template made from RNA.

As a result, there are hundreds of thousands of different proteins in the body, all with different forms and functions. They help build and control life at the chemical level.

The ribosome had resisted efforts to unveil its structure for four decades, but from 1999 to 2005 a number of groups revealed different aspects of the structure so that today we know exactly how the ribosome works at atomic resolution. The long period of obscurity is due to the size and complexity of this molecular machine – a tangle of ~54 proteins and three RNA strands (over 1500 bases) complicated every step needed to work out its structure. Not only was an understanding of the ribosome's mechanism only important scientifically, but the information can be used to design new antibiotics. Many useful antibiotics (e.g. aminoglycosides, tetracyclins, lincosamides, streptogramins, macrolides and aminocyclitols) exploit the subtle differences between bacterial ribosomes and our own to specifically kill bacteria. In fact, one of the laureates (Tom Steitz) has founded a company (Rib-X) to develop new antibiotics for serious drug-resistant infections based on the structure of bacterial ribosomes. In fact, all three of this year's laureates have generated 3D models of the ribosome that show how different antibiotics bind to stop protein synthesis in bacteria.

Ada Yonath

Ada E. Yonath (www.weizmann.ac.il/sb/faculty_pages/Yonath/home.html) is an Israeli citizen, born in 1939 in Jerusalem. She did her undergraduate degree and Masters at the Hebrew University of Jerusalem in chemistry and then went on to earn her PhD in X-ray crystallography in 1968 from the Weizmann Institute (with William Traub). After a couple of years of relatively unproductive postdoctoral appointments in the USA (Carnegie Mellon and MIT), she returned to the Weizmann Institute as a research scientist in the Chemistry Department and is currently Martin S. and Helen Kimmel Professor of Structural Biology and Director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly. Her interest in big crystallographic problems stemmed from her earliest days.



Ada Yonath. Photo courtesy of Ada Yonath.

Originally she worked on the crystal structure of peptides that mimicked collagen and got her first paper in *Nature* magazine (1969) by solving the structure of real collagen (Traub 1969).

In the 1970s, Ada Yonath decided her mission would be to crystallise the ribosome. This was met with scepticism from the scientific community as the sheer size of the task, to crystallise a complex with 50+ proteins and even more RNA, should have been daunting to anyone but, as James Watson stated in 1964, '...we cannot accurately describe at the chemical level how a molecule functions unless we know first its structure' (Watson 1964). Ada started her mission by first determining the structure of an initiation factor; a protein that helps jump-start protein synthesis by binding temporarily to the ribosome. But even that single protein proved difficult to purify in quantities sufficient for structural studies. In 1978, Yonath suffered a further setback: by then she had arranged to work at the Max Planck Institute for Molecular Genetics in Berlin, Germany, which had

undertaken a large research effort on ribosomes and which had the protein-purification equipment she didn't have in Israel. But a bicycle accident landed her in hospital and convalescence for several months. In that time she read a lot, not just about her field of research but also more widely. She read about polar bears and hot springs, among other things, but what impressed her was that polar bears hibernate all winter and preserve their ribosomes by aggregating them on membranes. She knew that ribosomes are extremely unstable and that for ribosomes to last over winter, polar bears had to do something to preserve them. She thought that the aggregation described by the paper might have been involved in their preservation and that it may, thus, be possible to make crystals. The second idea was to use ribosomes from bacteria that live at extreme temperatures (extremophiles) as, she reasoned, organisms that can survive near boiling water should have very stable ribosomes.

Once Ada arrived in Berlin as a visiting professor, she immediately decided to ask the institute's director (Heinz Günter Wittmann) if she could try to crystallise ribosomes, which littered the fridges and freezers of his lab. He said this was the dream of his life and gave her everything she needed.

It took Ada months of trying different solutions and crystallisation procedures (before the advent of commercial kits to do this) to get tiny crystals of the larger (50S) subunit of the ribosome from a bacterium (*Geobacillus stearothermophilus*), and another couple of years to get the first, very fuzzy X-ray crystallographic images. However, when she showed colleagues her results at a conference in 1980, she was treated like the village idiot. It was generally believed that something as big and dynamic as the ribosome could never be crystallised and that Ada's dream of polar bears and extremophiles was, in fact, a pipe dream. However, a few key people didn't scoff.

One was Wittmann, her mentor, and another was Sir John Kendrew, himself a Nobel laureate and then director of the European Molecular Biology Laboratory in Heidelberg. Kendrew and Perutz received the 1962 Nobel Prize for 'their studies of the structure of globular proteins' but more specifically for solving the phase problem in crystallography by adding heavy metals to protein crystals (more about that next month) that act as beacons in the diffraction patterns.

Kendrew helped ensure that Ada was able to continue to get beam-time for her X-ray diffraction studies, despite the high risk of failure and lack of funding. Then in 1985, Yonath produced crystals of the Bacillus 50S sub-unit good enough to yield diffraction patterns in which she could distinguish atoms down to 6 Å apart. This was pivotal as it proved to the world that it was possible to get high-resolution crystals of ribosomes. Wittmann and Yonath published their results in a series of papers from 1980 to 1987 in relatively specialised journals.

Even so, getting atomic resolution (<3 Å) proved difficult because the protein crystals tended to disintegrate after hours of exposure to intense X-rays. Another great idea Yonath had (from the polar bears?) was to freeze the tiny crystals she produced in liquid nitrogen to stabilise them. She enlisted the help of Hakon Hope, a crystallographer at the University of California, Davis, to establish the method. Considering the ubiquity of this technique today, she should be given a lot of credit. These results were published by Yonath (with Wittmann and Hope) in 1989 (Hope et al. 1989). With more stable crystals in hand, the next step was to determine how to create specific landmarks for phasing in the diffraction patterns, but because the ribosome is so large, Kendrew's method didn't work so Wittmann and Yonath tried to use clusters of heavy atoms instead. Even with these advances, by 1995 when the international ribosome conference took place in Victoria, Canada, her report on the 50S subunit was disappointing. Now other ribosome researchers were beginning to champ at the bit (Pennisi 1999).

In science, the tradition has always been to allow some latitude to researchers to see their research through to the end without too much interference. Because a sort of territorial claim had been staked by Yonath and Wittmann in 1980 on the ribosome, few others had been pursuing the structure. However, one rival effort had begun behind the Iron Curtain during the 1980s. Meeting as graduate students in Alexander Spirin's lab, Gulnara Yusupova and Marat Yusupov

embarked on their own pursuit of ribosome crystals at the Protein Research Institute in Pushchinoin, Russia in 1983. By 1987, they had produced crystals of the entire ribosome and of the smaller 30S subunit from the bacterium *Thermus thermophilus*, but it was impossible to solve the structure in Russia because of the lack of suitable X-ray beam-time. After the dissolution of the Soviet Union in 1989, the Yusupovs began collaborating with Dino Moras at the University of Strasbourg in France. There they produced very good quality crystals but the work bogged down in the early 1990s because of shortages of funding. So in 1996, the Yusupovs packed up their crystals and moved to California to work with Harry Noller at the University of California, Santa Cruz. Peter Moore and Tom Steitz at Yale had also started collaborating. They used NMR spectroscopy and neutron diffraction as well as X-ray crystallography to determine the structures of individual proteins of the ribosome. Meanwhile Joachim Frank (University of California, Santa Cruz, USA) and Marin van Heel (Fritz Haber Institute, Berlin) were publishing very rough structures of the ribosome using a technique called cryo-EM. Researchers were closing in from all sides and at the ribosome conference in Helsingor, Denmark, in June 1999, it was clear that the final solution was not going to come from Ada's lab.

In the next article we look at the final turns of the race for the ribosome and the contribution of the other two laureates.

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REFERENCES

Crick F. 1970, *Nature* **227**, 561-3.
Hope H., Frolow F., von Böhlen K., Makowski I., Kratky C., Halfon Y., Danz H., Webster P., Bartels K.S., Wittmann H.G. et al. 1989, *Acta Crystall. Sect. B* **45**, 190-9.
Pennisi E. 1999, *Science* **285**, 2048-51.
Traub W., Yonath A., Segal D.M. 1969,*Nature* **221**, 914-17.
Watson J.D. 1964, *Bull. Soc. Chim. Biol. (Paris)* **46**, 1399-425