Golden jubilee of the cracking of the genetic code

Beginning of an Era

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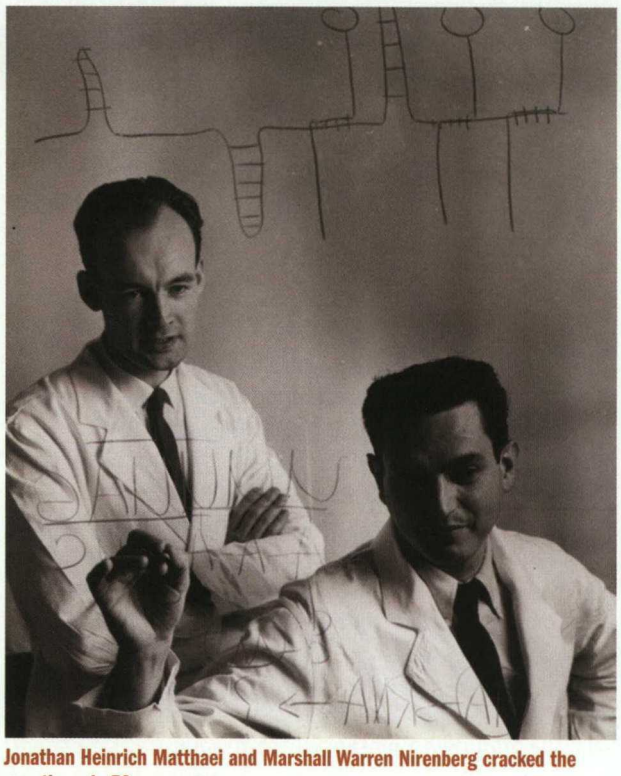
The current era of biochemistry was initiated by an experiment that changed the course of science

It was Saturday, 27th of May 1961, at 3 o'clock in the morning. Conducting research at the National Institutes of Health (NIH) in Bethesda, Maryland, Dr. Jonathan Heinrich Matthaei, working with Marshall Warren Nirenberg, placed polyuridylic acid into 20 different test tubes. All of the 20 test tubes contained also a cell-free extract from the bacteria *Escherichia coli*; in addition Dr. Matthaei added to each of the 20 different test tubes one of the 20 different radioactively labled amino acids known at that time. With this experimental setup he was first to decipher that the UUU triplet was the codon for the amino acid phenylalanine. Thus, 2011 saw the 50th anniversary of Nirenberg and Matthaei's classic experiment, a breakthrough in cracking the genetic code! It was one of the most important discoveries in the history of biochemistry and molecular biology, bringing us closer to understanding the rules of the genetic code.

A few weeks after the discovery, in August 1961, Nirenberg publicly announced the results during the 5th International Congress of Biochemistry in Moscow, organized by the Biochemistry Association of the Soviet Union under the auspices of the International Union of Biochemistry. Nirenberg and Matthaei's paper "Comparison of Ribosomal and Soluble *E. coli* Systems Incorporating Amino Acids into Protein" was in fact presented twice during the conference.

Matrix synthesis

Previously, having taken on a research post at the Metabolic Enzymes Department at the NIH, in the summer of 1960 Nirenberg observed that protein synthesis in a cell-free extract relied on a DNA matrix, and its deciphering required messenger RNA. Other researchers studying protein synthesis and trying to crack the genetic code came to similar conclusions. In the autumn the same year, Nirenberg was joined at the NIH by Matthaei, a German physiologist studying protein synthesis. They worked on *E. coli* systems until December 1960, and demonstrated that RNA stimulates protein synthesis. Their work was similar to research conducted by Heinz Fraenkel-Conrat from the Virology Laboratory at the University of California, Berkeley. In the spring of 1961, Nirenberg visited Fraenkel-Conrat at his laboratory in order to get some information on the tobacco mosaic virus (TMV) system. At the same time Heinrich Matthaei started in Bethesda the experiments researching the activity of single-stranded homo-oligonucleotides (poly-A and poly-U) and double-stranded hetero-polynucleotides (poly-AAU, poly-AAAAU). Each synthetic polynucleotide was checked in the presence of 19 unmarked amino acids and a single radioactive amino acid. On the morning of 27 May 1961, Matthaei made a fascinating observation:



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Jonathan Heinrich Matthaei and Marshall Warren Nirenberg cracked the genetic code 50 years ago

the results indicated that using a synthetic polynucleotide comprising poly-U uridine nucleotides only caused it to make proteins composed entirely of radioactive polyphenylalanine. Interestingly enough, Nirenberg received the news of Matthaei's breakthrough results while he was visiting Fraenkel-Conrat's laboratory in Berkeley.

Standing ovation

The day before the August 1961 Congress in Moscow, Nirenberg met James Watson and he proudly showed his poly (U) experimental results to Watson, who was rather skeptical about the discovery.

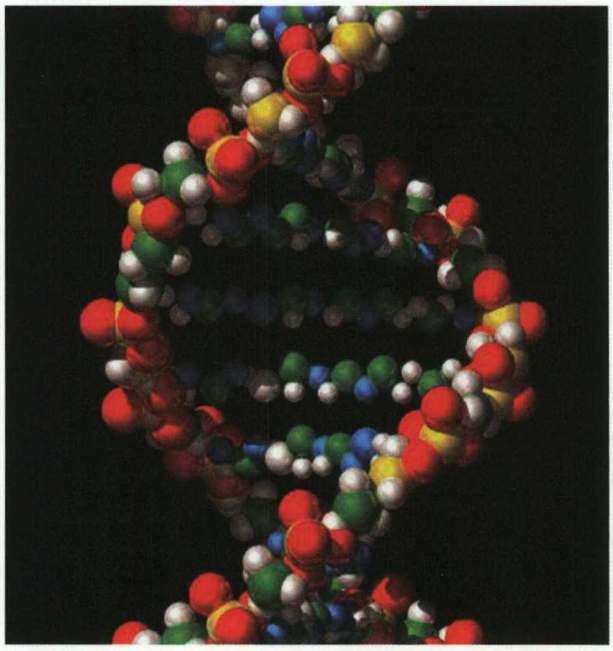
When Nirenberg made his first 15-minute public presentation to just about 35 researchers in a small back room; the presentation and the results went almost unnoticed. Fortunately, news of Nirenberg's spectacular results percolated through to Francis Crick, who convinced the organizers to repeat the Nirenberg lecture in a larger auditorium during the main symposium on nucleic acids. Nirenberg included specific data in his second lecture. The audience was electrified by the results, and gave the presentation a standing ovation.

The experiment with synthetic RNA in a cell-free system indeed marked a breakthrough, although it was also just the beginning of the long journey towards deciphering the entire genetic code. Researchers came to face many new challenges; the most important questions concerned the bases comprising each codon and the sequence of those bases in specific codons. Answering those questions later turned out to require even more intensive research.

The next breakthrough came in 1964 and 1965, when Dr. Philip Leder, one of Nirenberg's colleagues at NIH, devised a method to define the sequence of nucleotides in codons. This technological advance accelerated the process of assigning a code to individual amino acids. This finally lead to the identification of all codons soon thereafter. The achievements of Har Gobind Khorana in the chemical synthesis of oligonucleotides was of great help in finally assigning to all amino acids their corresponding three letter genetic code.

Back in 1953, when James Watson and Francis Crick discovered the structure of the DNA molecule to be a double helix with a sequence of bases on one strand matching the sequence on the other strand, by what is now known as Watson-Crick base pairing, they proposed that these base sequences contain a code that carries the genetic information. The actual discovery of this genetic code in the early 1960s kicked off the era of genetic research which continues until the present time.

Receiving the Nobel Prize in 1962, Francis Crick said: "We are coming to the end of an era in molecular biology. If the DNA structure was the end of the beginning, the discovery of Nirenberg and Matthaei is the beginning of the end."



NASA Marshall Space Flight Center (NASA-MSFC)

Watson and Crick, discoverers of the double helix, had asserted in 1953 that the DNA molecule must contain some code for protein production

Beginning of the end of the road

In 1966, the genetic code table containing 64 trinucleotide codons defining the code for the 20 different amino acids had been solved. Today, there are actually 22 different amino acids known as the building blocks for proteins. The determination of the genetic code has permitted researchers to make new discoveries and to introduce new techniques to molecular biology, such as DNA sequencing, recombinant DNA technology, as well as initiating the human genome project and proteomics research. In 1968, Nirenberg, Khorana (developer of the method for the chemical synthesis of nucleic acids) and Robert W. Holley (the first scientist to determine the nucleotide sequence of a transfer RNA) received the Nobel Prize in Physiology and Medicine. The work done in these three laboratories helped us to understand how the sequence of nucleic acid building blocks will eventually be translated into amino acids and then into proteins.

Nirenberg passed away on 15 January 2010. Heinrich Mathaei left the United States after his postdoctoral period with Marshall Nirenberg at NIH, to assume a position as a department chairman at the Max-Planck Institute for Experimental Medicine in Göttingen, Germany. He is currently still living in Göttingen. ■

Further reading:

Barciszewski J., Markiewicz W. (2011). Kwasy nukleinowe. Kod genetyczny [Nucleic Acids - The Genetic Code]. In: *Polskie i światowe osiągnięcia nauki* [Polish and World Achievements in Science]. Ed. Bylicki A., pp. 47-92, www.fundacjarozwojunauki.pl.

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