History of Science

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2011: 50th Anniversary of the Discovery of the Genetic Code

Volker A. Erdmann and Jan Barciszewski*

genetic code · history of science · molecular biology

Dedicated to Professor Alexander Rich

1. Introduction

This year we are celebrating the 50th anniversary of the discovery of the genetic code, which has turned out to be universal for all living organisms on earth. In 1961 Marshall Warren Nirenberg and Johann Heinrich Matthaei set up an in vitro translation system composed of purified bacterial ribosomes and a fraction of low-molecular-weight RNA, and demonstrated that polyuridilic acid codes for polyphenylalanine. This was the first experiment giving insight into the genetic code. In this Essay we summarize some achievements that led to the discovery of the genetic code, key experiments for the discovery of the genetic code, and developments after the determination of the genetic code. We conclude by discussing the impact of this for current molecular biology and medicine.

2. Achievements Leading to the Discovery of the Genetic Code

The question of a mechanism that organisms inherit in order to pass along their traits (characteristics) from one generation to the next generation was always of great interest in biology. This interest can be traced back to the 19th century, when Gregor Mendel established his basic rules of heredity, which were later confirmed by E. Tschermak, H. de Vries, and C. Correns. Mendel demonstrated the involvement of some factors, which are now called genes, and defined the terms recessive and dominant traits. In fact, it was at that time that the field of genetics was born. Mendelian genetics permits plant breeders to develop stronger and weed- and pest-resistant hybrid plants.[1] In 1913 it could be demonstrated that genes are irreducible units of information lined up on the chromosomes, the threadlike bodies in the nucleus of the cells.[2] In the following years, more information about genes and their roles for living organisms was collected.[2]

[*] Prof. Dr. V. A. Erdmann
Institute of Chemistry and Biochemistry, Free University of Berlin
Thielallee 63, 14195 Berlin (Germany)
E-mail: erdmann@chemie.fu-berlin.de
Prof. Dr. J. Barciszewski
Institute of Bioorganic Chemistry of the Polish Academy of Sciences
Noskowskiego 12, 61-704 Poznan (Poland)

The important step on the way to the discovery of the genetic code was the identification of deoxyribonucleic acid (DNA) as the carrier of all genetic information. It was Oswald Avery who discovered first that the substance important for inheritable changes in disease-causing bacteria was DNA. Together with Colin MacLeod and Maclyn McCarty, he suggested that DNA, not proteins, was responsible for transferring the genetic information. In fact, they showed that DNA was the molecule of heredity.

After Avery, MacLeod, and McCarty's findings were published, many scientists directed the focus of their studies to this new class of molecules, namely, nucleic acids.[3] In the 1950s Alfred Hershey and Martha Chase confirmed that DNA was indeed the genetic material,[4] and it was Erwin Chargaff[5] who determined that in all species there is 1:1 ratio of the bases A to T and G to C, which are the four basic building blocks of DNA.

In their famous publication in 1953 James D. Watson and Francis Crick described the structure of DNA as a double helix,[6] consisting of two long, coiled phosphate sugar chains joined by what we now refer to as Watson–Crick base pairs A–T and G–C. Watson and Crick also suggested already at that time that the bases could represent a code defining the order in which amino acids should be linked to form proteins. Also, in this publication they stated that the specific base pairing they had envisioned immediately suggested a possible mechanism for copying the genetic material.[6]

After the model for the DNA structure had been proposed a central problem in molecular biology was to understand the nature of the genetic code. How could information encoded by four DNA nucleotides be converted into a functional sequence of twenty different amino acids in proteins?[7]

In 1954 Marianne Grunberg-Manago and Severo Ochoa started to work on an enzyme involved in nucleic acid synthesis. The enzyme, polynucleotide phosphorylase, permitted the first in vitro synthesis of higher-molecular-weight biological nucleic acids.[8] Also at this time, Paul Zamecnik became interested in identifying the components required for protein biosynthesis. Already in 1953 he succeeded in making the first cell-free system capable of peptide bond formation using ^{14}C-labeled amino acids. Together with Elizabeth Keller and Mahlon Hoagland, they demonstrated that the initial step in protein synthesis is the activation of amino acids by the formation of aminoacyl adenylates from the amino acids and ATP.[9]

Using the cell-free system, P. Zamecnik and M. Hoagland found that the RNA in a particular cytoplasmic fraction became labeled with ^{14}C-amino acids and that the labeled RNA was subsequently able to transfer these amino acids to a microsomal protein fraction. The transfer was also dependent upon guanosine triphosphate (GTP). From these results they concluded that the RNA, later named transfer RNA or tRNA, functions as an intermediate carrier of amino acids in protein synthesis.[9]

In other very important experiments Alexander Rich and Robert Davies were able to show already in 1956 that single strands of RNA can join together (hybridize) to form double-stranded RNA.[10,11] This discovery came only three years after James Watson and Francis Crick had described the DNA structure as a double helix and how inherited genetic information could potentially be stored and transmitted from one generation to the next.[6] Currently nucleic acid hybridization methods are routinely used to identify, isolate, manipulate, replace, and inhibit genes in living systems. Indeed, the early proposal by Alexander Rich that nucleic acids are able to hybridize led to the key technique used in the field of genetic engineering and biotechnology.

After the DNA structure had been proposed, the decoding of the genetic information was a very exciting topic. Interestingly, the theoretical physicist George Gamow was actually the originator of the well-known "Rnatie Club" (whose slogan was: "Do or die, or don't try"), in which each member would put forward his ideas on how the nucleotide bases are finally translated into proteins in the cells.[12] The club had twenty hand-picked members, one for each amino acid (for example, Crick–tyrosine, Watson–proline, Rich–arginine, Chargaff–lysine, etc.) and each wore a tie marked with the symbol of that amino acid. Among other things, they debated how many bases would be required to make up the genetic code.[13]

The Rnatie Club members knew at that time that there were the four bases: guanine, cytosine, adenine, and thymine. So, if there were two bases in each codon, then there would be the possibility of only 4^2, in other words 16, unique combinations of bases, or 16 amino acids. But since there were 20 known amino acids at that time, they had to assume that there would have to be at least three bases for each codon, and that this requirement could only be met by $4^3 = 64$ possible combinations. The club members were very active in the 1950s but did not manage to break the code. Interesting enough, Marshall Nirenberg was not a member of the Rnatie Club.

However, in a famous letter send in 1955 to the club, Francis Crick predicted the existence of small adaptor RNA molecules that would be able to carry their own amino acids and that they would interact with the messenger RNA template in a position such that the polymerization of the proteins would be permitted. These adaptors, larger than predicted by Crick, are actually the tRNAs discovered in 1958 by P. Zamecnik and M. Hoagland.[14]

After the double helix, the central dogma of molecular biology[15,16] and the semiconservative replication[17] entered the vocabulary of molecular biologists. It was in 1958 that the ribosomes were discovered by Georg E. Palade.[18] In prokaryotes, they are composed of more than 50 individual proteins and three large ribosomal RNAs. In the cell, they are responsible for peptide bond formation, and they are also the target of the majority of antibiotics. These earlier biochemical and physical investigations provided a quite reasonable picture of the overall structure, biochemical reactions, and molecular interactions that take place on ribosomes when proteins are made.[19,20]

As it turns out, the synthesis of the polypeptide chain is an extremely complex reaction. It consists of three different phases termed initiation, elongation, and termination. These, in turn, require several kinetically distinct steps, usually associated with different conformational states of the ribosomes. Conformational changes of the ribosome during protein synthesis are usually controlled by translation factors and the hydrolysis of GTPs. Very promising progress has been made recently in this field through a combination of cryo-electron microscopy and X-ray crystallography.[21]

A few years after the DNA structural model from Watson and Crick appeared, Matthew Meselson and Franklin Stahl came up with a unique method for distinguishing new and old DNA during replication.[17] In their experiment they first grew *Escherichia coli* cells in a culture medium containing only the heavier isotopic form of nitrogen, N^{15}. After one generation, the cells were transferred to a growth medium containing only the lighter form of nitrogen, namely the N^{14} form. Meselson

Volker A. Erdmann, born 1941 in Stettin, Germany, received his BA (1963) and MSc in Biochemistry (1966) from the University of New Hampshire in Durham (USA). In 1968 he received his PhD from the Max-Planck-Institute for Experimental Medicine in Göttingen and the University of Braunschweig (Germany) with Friedrich Cramer. After postdoctoral fellowships in Göttingen (1968–1969) and the University of Wisconsin, Madison, with Masayasu Nomura (1969–1971) he joined the department of Heinz-Günter Wittmann at the Max-Planck-Institute for Molecular Genetics in Berlin (1971–1980) as an independent group leader. From 1980 until 2009 he held the Chair for Biochemistry at the Free University of Berlin. His research interests are protein biosynthesis, RNA structures and functions, and the development of RNA technologies for biotechnology and molecular medicine.

Jan Barciszewski received his PhD in 1974 in organic chemistry from the Adam Mickiewicz University in Poznan, Poland. Since 1993 he has been Professor of Biology at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznan. He has worked abroad at the Institute of Molecular Biology in Moscow, Aarhus University (Denmark), Zurich University (Switzerland), the National Cancer Center in Tokyo (Japan), the Institute of Molecular and Cellular Biology in Strasbourg (France), and at the Free University of Berlin with Volker A. Erdmann. His research focuses on the structure and function of nucleic acids and their interactions with proteins, catalytic RNAs, and the development of nucleic acid tools for the treatment of brain tumors and other diseases.

and Stahl then isolated the DNA after different growth generations and simply analyzed them in cesium chloride centrifugation experiments. From these experiments they were able to show very convincingly that replication takes place according to the semiconservative mechanism; that is, with each cell division a cell receives one "old" and one "new" DNA strand in their chromosomes.[17] By 1960 it was clear that DNA itself is not directly involved in protein synthesis, and this is how DNA is transcribed (copied) to a single-stranded messenger RNA, called mRNA.[22]

But the key question still remained: Which sequence of bases specifies which amino acid, and thus what is the genetic code?

The important role of messenger RNA (mRNA) was clarified by François Jacob, Jacques Monod, and Sydney Brenner in 1961. In another famous experiment in the same year Jacob and Monod described the regulatory mechanisms involved in the translation of mRNAs into proteins and they were also able to formulate what came to be known as the "central dogma of modern biology", which simply states that information flows from DNA to RNA to protein.[23]

3. Key Experiments in the Discovery of the Genetic Code

In 1959 Marshall W. Nirenberg at the National Institutes of Health (NIH) began experiments aimed at understanding how DNA information is expressed as proteins. In fact he thought that amino acids were encoded into proteins with the help of nucleic acids, but he did not know whether DNA or RNA was involved, and of course, at that time he did not know if a code existed. Therefore the first question was: Does DNA or RNA stimulate amino acid incorporation into proteins in cell-free extracts?

Leon A. Heppel, Maxine Singer, and others[24] had synthesized RNA composed of chains of single repeating bases. Using polynucleotide phosphorylase, they had synthesized poly(U), an RNA molecule consisting entirely of uridine. This enzyme originally discovered by Grunberg-Manago and Ochoa[7] played a key role in answering questions concerning the genetic code.[24]

In the historic poly(U) experiment, which was designed by Nirenberg and Matthaei (Figure 1) and which was performed by Heinrich Matthaei on Saturday, May 27, 1961, at 3 a.m., poly(U) was incubated with an *Escherichia coli* extract and C^{14}-labeled phenylalanine. The results were clear: poly(U) codes for phenylalanine (Figure 2) Subsequent experiments confirmed that poly(U) programs the synthesis of polyphenylalanine and that the code is composed of triplets; that is, the three-nucleotides UUU is the template for phenylalanine. In a similar way poly(C) produces polyproline and CCC is the code for that amino acid. In the presence of the *E. coli* crude extract and purified RNA, amino acids specified by groups of bases called codons were incorporated into proteins. Thus, the relationship between the RNA and its nucleotide sequence, which encoded the amino acid sequence of the generated protein product, became obvious.[24,25]

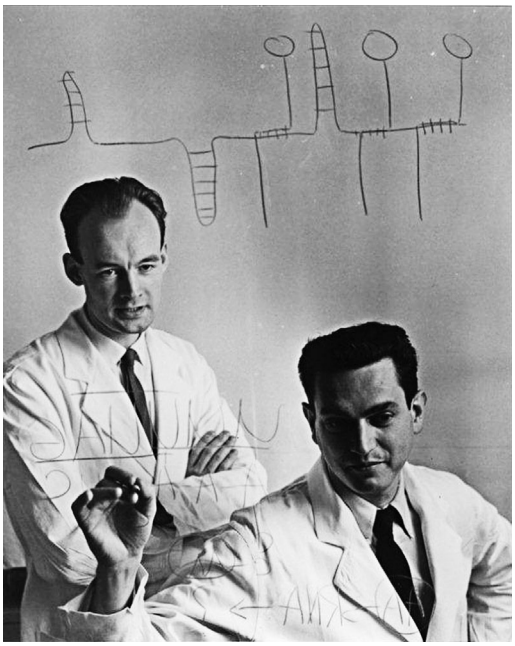


Figure 1. Marshall Warren Nirenberg (right) and Johann Heinrich Matthaei are shown designing their in vitro translation experiments to solve the genetic code. This picture, taken in 1962 at the National Institutes of Health in Bethesda, MD, was kindly supplied by the National Institutes of Health, USA.

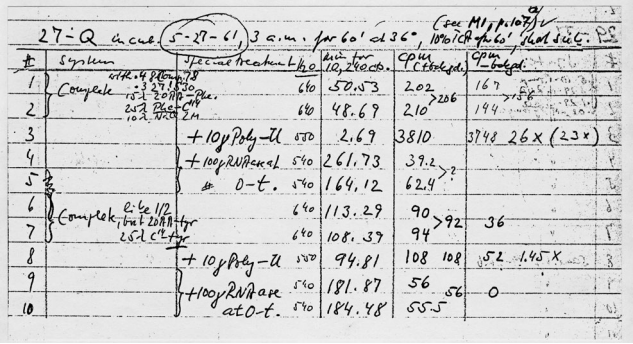


Figure 2. Copy of Heinrich Matthaei's laboratory book page on which the experimental conditions are described for the well-known poly(U) experiment performed on May 27th, 1961 at 3 a.m. at the National Institutes of Health, Washington DC, USA. The experiment led to the conclusion that the sequence UUU codes for the amino acid phenylalanine during ribosome-dependent protein synthesis. The picture was kindly supplied by Heinrich Matthaei, Göttingen, Germany.

Interestingly, Nirenberg and Matthaei also showed that single-stranded poly(U), but not double- or triple-stranded poly(U)–poly (A) helices can function as mRNA. In fact this result and the previously mentioned prediction from Alexander Rich were the first RNA antisense experiments ever performed.

The key step of the code discovery was precipitation of homopolypeptides from trichloroacetic acid (TCA). This is possible with polyphenylalanine and polyproline, which are not soluble in TCA and easily recovered as precipitates on filters. However, polylysine, which is soluble in TCA, can only

be precipitated with a trichloroacetic–tungstate (TCA/W04) solution. In other words, in a poly(A) ^{14}C-lysine experiment, TCA would not have precipitated the polylysine and therefore the poly(A)-directed polylysine synthesis would not have been detected.

By 1966, 64 potential trinucleotide codons had been identified. Since RNA is constructed from four types of nucleotides, there are 64 possible triplet sequences ($4 \times 4 \times 4$). Three of these possible codons specify the termination of the polypeptide chain (stop codons). That leaves 61 codons to specify only 20 different amino acids. Therefore, most of the amino acids are represented by more than one codon.[18,24] It was shown that the remaining three codons are punctuation marks indicating the end of a gene (Figure 3). In subsequent experiments, M. Nirenberg showed that the same code is used in other species and suggested that it occurs in all species on earth.

The Genetic Code

First Position (5′ end)	Second Position				Third Position (3′ end)
	U	C	A	G	
U	Phenylalanine	Serine	Tyrosine	Cysteine	U
	Phenylalanine	Serine	Tyrosine	Cysteine	C
	Leucine	Serine	Stop	Stop	A
	Leucine	Serine	Stop	Tryptophan	G
C	Leucine	Proline	Histidine	Arginine	U
	Leucine	Proline	Histidine	Arginine	C
	Leucine	Proline	Glutamine	Arginine	A
	Leucine	Proline	Glutamine	Arginine	G
A	Isoleucine	Threonine	Asparagine	Serine	U
	Isoleucine	Threonine	Asparagine	Serine	C
	Isoleucine	Threonine	Lysine	Arginine	A
	Methionine	Threonine	Lysine	Arginine	G
G	Valine	Alanine	Aspartic Acid	Glycine	U
	Valine	Alanine	Aspartic Acid	Glycine	C
	Valine	Alanine	Glutamic Acid	Glycine	A
	Valine	Alanine	Glutamic Acid	Glycine	G

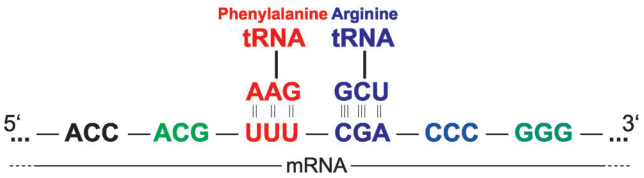


Figure 3. A summary of the genetic code as it was established in the 1960s. Below the table is an example of a partial mRNA sequence and the codon–anticodon interaction of a phenylalanine-tRNA^{Phe} and an alanine-tRNA^{Ala} with the mRNA. This graphic was kindly supplied by Jörn V. Erdmann, Berlin.

By 1965, Nirenberg with the help of his NIH colleagues Merton Bernfield, Leon Heppel, Philip Leder, and Maxine Singer, had become the first to complete the sequencing of the genetic code. The language of DNA was understood and the genetic code could be expressed in a chart. By looking up the sequence of nucleotide bases, the reader can identify the corresponding amino acid. In order to read the code, one selects a letter from the left column, the top row, and the right column. This combination represents an mRNA codon (Figure 3).

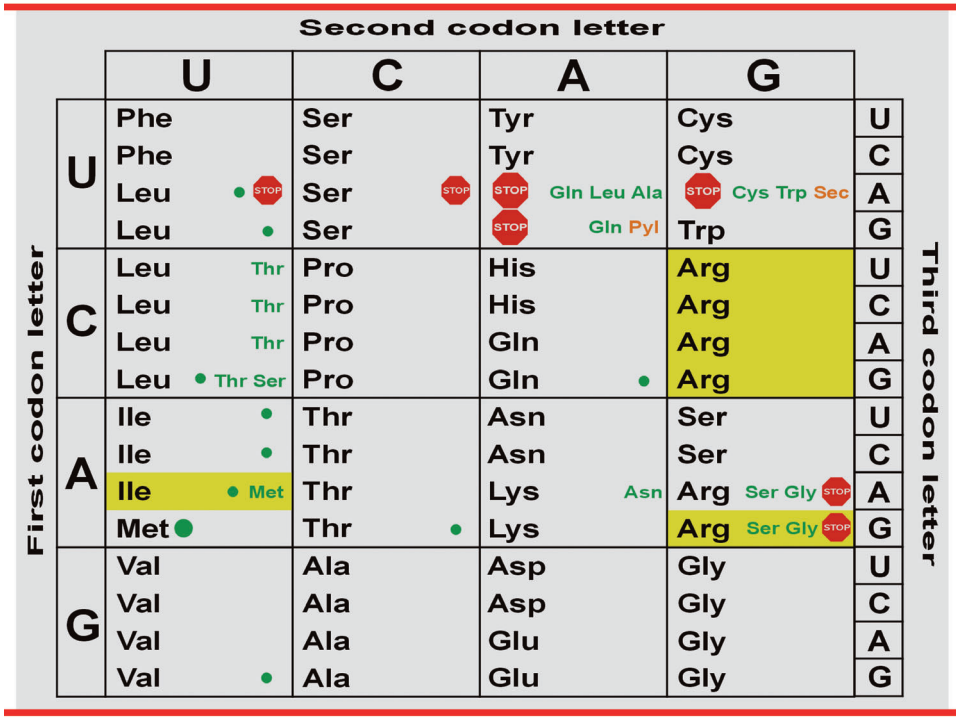
In early 1961, Peter Lengyel and Joseph Speyer, while working in Severo Ochoa's laboratory, started to work with cell-free protein synthesis systems. Their idea was that synthetic polyribonucleotides, of known base composition and synthesized with polynucleotide phosphorylase, would act as mRNAs and direct the incorporation of amino acids into proteins depending upon the polyribonucleotide base composition.[26] The hypothesis was very successful, and the laboratories of Ochoa and Nirenberg were engaged in a highly competitive race to solve the mystery of the genetic code. In the early 1960s Har Gobind Khorana confirmed and expanded upon much of many aspects of the genetic code by chemically synthesizing deoxyribonucleotides of known base sequence, and demonstrated that they directed the incorporation of specific amino acids into proteins.[27]

The genetic code is unambiguous, which means that each codon specifies one amino acid only. On the other hand, it is also degenerate; in other words, one amino acid may be specified by more than one codon. In most cases sufficient coding is performed by the first two bases, the third base, or wobble base, plays a minor role. For instance, the four codons that specify glycine (GGU, GGC, GGA, and GGG) all start with GG (see Figure 3). Codons with a similar sequence specify amino acids with similar chemical properties. The codons that specify threonine differ from those specifying serine by their 5′ nucleotide. The codons for aspartate and glutamate differ only by their 3′-position. Codons that contain a pyrimidine in the middle generally specify for a hydrophobic amino acid. Thus, mutations of the 5′- or 3′-positions of these codons lead to a substitution of chemically similar amino acids. The STOP codons trigger the termination of translation by the ribosome. The codon AUG specific for methionine can also be used as a start codon.[28]

Human mitochondrial DNA encodes only 22 tRNA species, which are used for the translation of mitochondrial mRNAs. This is accomplished by a wobble in which uridine of the anticodon in the tRNA can pair with any of the four bases in the third codon position of the mRNA, allowing four codons in the mRNA to be recognized by a single tRNA. The genetic code was once thought to be universal and frozen, but later on multiple codon reassignments in nuclear and mitochondrial genomes were found.[29] These changes include stop-to-sense, sense-to-sense, and sense-to-stop codon substitutions. A major reassignment in mitochondria is the recoding of UGA to tryptophan implemented by a tRNA^{Trp} with a mutation in its anticodon. Also, the mitochondria of several yeast species reassigned the AUA codon from isoleucine to methionine by the abnormal recognition of AUA by tRNA^{Met}.[29]

As evident in Figure 4, the genetic code has undergone a series of expansions.[30,31] Thus, the genetic code has opened the way not only for the expansion of molecular biology, but also to the birth of modern biotechnology, to the Human Genome Project, and to new discoveries that have deepened our understanding of how life is sustained and changed from generation to generation.

THE GENETIC CODE



Exceptions to the standard code. The numbers in brackets refer to the translation tables used in GenBank/EMBL databases listed below

UUA	terminator in *Thraustochytrium* mitochondrial code (23), initiation codon in protozoan mitochondrial code and *Mycoplasma*/*Spiroplasma* code (4),
UUG	initiation codon in standard (1), bacterial (11) and some mitochondrial codes (4,5, 13),
UCA	terminator in *Scenedesmus obliquus* mitochondrial code (22),
UAA	Gln in ciliate nuclear code (6); Tyr in alternative flatworm mitochondrial code (14); Pyl (pyrrolysine) in Archaea (Methanosarcinaceae) decoded by Pyl-tRNA,
UAG	Gln in ciliate nuclear code (6,15); Leu in Chlorophyceae, and *Scenedesmus* mitochondrial codes (16,22),
UGA	Trp in mitochondrial codes (2,3,4,5,9,13,14,21); Cys in euplotid nuclear code (10); Sec (selenocysteine) depends on a presence of SECIS (SElenoCysteine Insertion Sequence) element in mRNA,
CUU	Thr in yeast mitochondrial code (3),
CUC	Thr in yeast mitochondrial code (3),
CUA	Thr in yeast mitochondrial code (3),
CUG	Thr in yeast mitochondrial code (3); in alternative yeast mitochondrial code (12); initiation codon in standard (1) bacterial (11) and some mitochondrial codes (4,12),
AUU	initiation codon in bacterial (11) and some mitochondrial codes (2,4,5,23),
AUC	initiation codon in bacterial (11) and some mitochondrial codes (2,4,5),
AUA	Met in mitochondrial codes of vertebrates (2), yeast (3) and some invertebrates (5,13,21); initiation codon in bacterial (11) and some mitochondrial codes (2,3,4,5,13),
AAA	Asn in flatworm (9,14,21) and echinoderm (9) mitochondrial codes,
AGA	terminator in vertebrate mitochondrial code (2); Gly in ascidian mitochondrial code (13); Ser in mitochondrial codes (5,9,14,21),
AGG	terminator in vertebrate mitochondrial code (2); Gly in ascidian mitochondrial code (13); Ser in mitochondrial codes (5,9,14,21),
GUG	initiation codon in bacterial (11) and some mitochondrial codes (2,4,5)

Table of the genetic code. Initiation and termination codons are designated by green dots and STOP signs, respectively. Exceptions to the standard genetic code are shown in the right column in green (standard amino acids), orange (non-standard amino acids) and small symbols (initiation and termination codons). Yellow background indicates codons not used in some organisms. In the standard genetic code initiation and terminators codons are marked with large signs.

Amino acids: Ala-Alanine, Arg-Arginine, Asn-Asparagine, Asp-Aspartic acid, Cys-Cysteine, Gln-Glutamine, Glu-Glutamic acid, Gly-Glycine, His-Histidine, Ile-Isoleucine, Leu-Leucine, Lys-Lysine, Met-Methionine, Phe-Phenylalanine, Pro-Proline, Pyl-Pyrrolysine, Sec-Selenocysteine, Ser-Serine, Thr-Threonine, Trp-Tryptophan, Tyr-Tyrosine, Val-Valine.

GenBank translation table numbers. 1. Standard code; **2**. Vertebrate mitochondrial code; **3**. Yeast mitochondrial code; **4**. Mold, protozoan, and coelenterate mitochondrial code and the Mycoplasma/Spiroplasma code; **5**. Invertebrate mitochondrial code; **6**. Ciliate, dasycladacean and Hexamita nuclear code; **9**. Echinoderm and flatworm mitochondrial code; **10**. Euplotid nuclear code; **11**. Bacterial and plant plastid code; **12**. Alternative yeast nuclear code; **13**. Ascidian mitochondrial code; **14**. Alternative flatworm mitochondrial code; **15**. *Blepharisma* nuclear code; **16**. Chlorophycean mitochondrial code; **21**. Trematode mitochondrial code; **22**. *Scenedesmus obliquus* mitochondrial code; **23**. *Thraustochytrium* mitochondrial code. (Source: http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi)

Figure 4. The current version of the genetic code table in which all variants of the coding rules so far determined are included. The table is taken from the recent publication by Szymanski and Barciszewski[31] and with the kind permission of the journal *Acta Biochimica Polonica*.

4. Developments after the Determination of the Genetic Code

The seminal experiment that established the biological importance of accurate tRNA aminoacylation for the correct translation of the genetic code was performed in 1962 by Francois Chapeville.[32] In a series of experiments he showed that the coding properties of the adaptor is not determined by the amino acid it carries but by the interaction of the aminoacylated tRNA with the mRNA template. This was demonstrated by the observation that both cysteinyl-$tRNA^{Cys}$ and alanyl-$tRNA^{Cys}$ (obtained by reduction of the cysteinyl residue on $tRNA^{Cys}$ with Raney nickel) are amino acid donors in a ribosomal polypeptide synthesis system programmed with poly(UG) for cysteine incorporation.[32]

The first tRNA to be sequenced was the alanine-specific $tRNA^{Ala}$, which was reported by Robert Holley and his co-workers in 1965.[33] In the race for the first tRNA to be sequenced Hans Zachau and his co-workers came in as a close second, perhaps because their sequencing efforts had been hampered by the fact that their $tRNA^{Ser}$ preparation actually contained two specific tRNA sequences.[34] The third tRNA ($tRNA^{Phe}$) was sequenced by Tom RajBhandary in 1967, while he was working as a postdoctoral fellow in Khorana's laboratory.[35] The sequencing work of $tRNA^{Phe}$ by RajBhandary is especially worth mentioning, because it was actually the first tRNA whose three-dimensional structure was solved by X-ray crystallography—by Alexander Rich and co-workers in Cambridge, USA,[36] and by Aaron Klug and co-workers in Cambridge, England.[37] These studies revealed that the secondary cloverleaf structure of $tRNA^{Phe}$ is folded to form an L-shaped three-dimensional structure. Later structural studies with other specific tRNAs have confirmed the L-shaped three-dimensional structure to be of universal nature.

The first indications that the structure of ribosomes could be studied by X-ray crystallography came from the work of Ada Yonath and her collaborators in Berlin in 1980, when they crystallized ribosomes for the first time.[38] The first crystals obtained were those of the 50S ribosomal subunits from *Bacillus stearothermophilus,* but the methods for solving the structure of such a large ribonucleoprotein complex were not available at that time.[20] As a matter of fact, it took more than twenty years before the ribosomal structure could be elucidated in the laboratories of A. Yonath,[39] T. Steitz,[40] V. Ramakrishnan,[41] and H. Noller.[42] The recent high-resolution structures yield a wealth of valuable information concerning not only the structure, but also the function of the ribosome. Thus, it is now possible to locate the different tRNAs at the ribosomal A-, P-, and E-sites, which were discovered by K. Nierhaus and his co-workers,[43] and how they interact with the codons on the mRNAs.[44] For a very detailed summary of the developments leading to the discoveries of protein synthesis, see the book *Experimentalsysteme und epistemische Dinge* written by Hans-Jörg Rheinberger.[45] It is unfortunate that this book has been written only in German and that an English version has not yet been published; we are certain that this book would be of great interest to many scientists interested in the discoveries leading to our current knowledge of how genes are expressed as proteins.

5. Conclusion

Solving the genetic code changed not only molecular biology, but also how we are living today and how we can imagine living in the future. Clearly the identification of the genetic code helped us to understand much better the cellular processes in living cells, the rules determining cellular growth, and how traits are passed on to future generations. Perhaps more importantly for medicine is the fact that we are now entering a new phase, called molecular medicine. Future developments in this area will lead to the highly promising fields of personalized medicine and regenerative medicine, in which each patient will eventually be treated according to his personal needs.

But also the economic influence of a project such as the Human Genome Project (HGP), initiated by the United States in 1988 with US$ 3.8 billion in financial support, has also depended on the genetic code. As summarized in the recent report "Economic Impact of the Human Genome Project" by Barttelle[46] it is clear that the initial investment for the sequencing of the human genome has more than paid off for the American society: In 2011 returns of nearly US$ 800 billion have been generated in the fields of molecular biology, biotechnology, and medicine. Part of this sum has also been used to pay the 310000 employees in this field nearly US$ 250 billion in salaries, and to pay, alone in the year 2010, US$ 3.7 billion in taxes to the government of the United States of America.

Clearly that crucial experiment conducted by Nirenberg and Mathaei 50 years ago are worth remembering as one of the classic experiments in biology.

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