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The 60th anniversary of the discovery of DNA secondary structure

Otkriće sekundarne strukture molekula DNK – 60-godišnjica

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Introduction

The year 2013 is the year of great anniversaries in molecular biology. In 1953 Watson and Crick published the model of DNA structure in the scientific journal "Nature", indicating the model of its self-replication as well [1, 2]. This was the final proof that DNA molecule contains genetic information. With this discovery, exactly 60 years ago, molecular biology became distinct scientific discipline and today it is one of the most dynamic fields of science. There is no doubt that completion of one of the largest and the most expensive scientific project of all times, deciphering the sequence of the human genome, is one of the milestones that marked the beginning of the 21st century. The International Human Genome Sequencing Consortium published the results of their project in "Nature" in 2001 [3]. The very next day the results of the work done by a group of scientists employed by the company "Celera" appeared in "Science" [4]. The complete sequence of the human genome with the assessment of the gene number was published in 2003 [5]. At the time, the estimation of the number of the protein-coding genes was 30,000. Since then every year we witness a new revision of the number of human genes. According to the last data, the number of protein-coding genes is slightly more than 20,000 [6]. Starting in 2003, the Encyclopedia of DNA Elements (ENCODE) project set out to map which parts of human chromosomes are transcribed, how transcription is regulated and how the process is affected by the way DNA is packaged in the cell nucleus. The results of this project, published in 2012, showed that 80.4% of the human genome displays some functionality in at least one of 147 different cell types analysed [7].

While deciphering the human genome and other species' genomes, the new scientific branch, genomics, was established. The object of study in genomics is the whole genomes of species. It is the opinion of the scientific community that genomics of all species will bring about the most progress in the everlasting human struggle against incurable diseases and ageing [8]. The most important discoveries that enabled the advances in molecular biology and the beginning of the genomic era will be presented in this article.

Discovery of the secondary structure of DNA molecule

In spring 1951, during a zoology congress, the American zoologist James Dewey Watson (born 1928) met the New Zealander Maurice Hugh Frederick Wilkins (1916–2004) who showed him diffraction images of DNA molecule. For young Watson, this experience was initiation into the research of the chemical structure of nucleic acids and proteins. By the end of 1952 Watson also met a British physicist and biologist Francis Harry Compton Crick (1916–2004). The two started working together on the 3-dimensional model of a DNA molecule. In no more than couple of months, making molecular models based on characteristics of diffraction images of DNA, Watson and Crick established that a DNA molecule consisted of two antiparallel polynucleotide strands joined together by hydrogen bonds [9].

On April 25, 1953 in its 171st volume, the journal "Nature" published the article "Molecular structure of nucleic acids – A Structure for Deoxyribose Nucleic Acid" [1]. It was known at that time that DNA is the carrier of hereditary information. This was mainly due to the work of Avery et al. [10] who investigated the transformation of *Pneumococcus* type III bacteria and the horizontal gene transfer in this species. Therefore, it is not surprising that this publication was met by the utmost interest of the scientific community as soon as it ap-

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peared. Watson and Crick published their discovery of the DNA molecule structure based on the double stranded right-handed spiral model on only one page with 6 cited references. According to their original double helix model, purine and pyrimidine bases are facing each other on the inside of the molecule, stacked one on another (due to hydrophobic interactions) while phosphate groups are turned to the outside. The backbone of the molecule is formed by pentose sugar and phosphate groups (Figure 1). Since the diameter of the helix is the same along the length of the molecule, purine base in one strand is facing the pyrimidine base in another one. This principle designated as the complementary base pairing rule states that adenine (A) and thymine (T), respectively cytosine (C) and guanine (G) form hydrogen bonds and make base pairs with the same geometry [1].

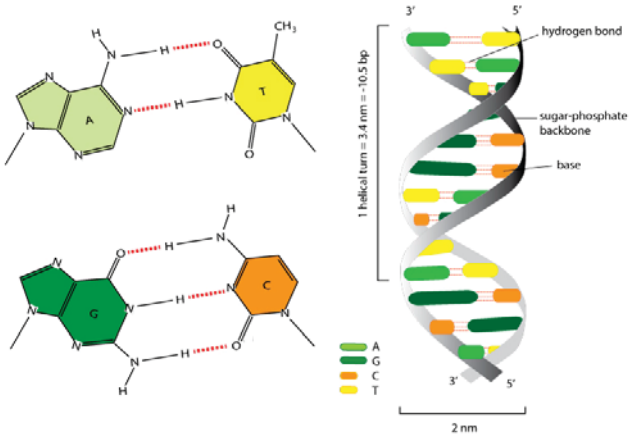

Fig. 1 – Complementary base pairs (left) and secondary structure of a DNA molecule (right)

Relying on the model of DNA secondary structure they proposed, Watson and Crick reflected on the possible way of the replication of DNA molecule. Therefore, the scientists emphasized in their article: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" [1].

At the time of this discovery, Watson was only 25 years old, and he won the Nobel Prize at the age of 34. Even today, in his late years, Watson is active and certainly is one of the greatest authorities in molecular biology. Last years have been marked by his controversial statements about the influence of genes on the human nature [11].

Discovery of the secondary structure of a DNA molecule still captures the interest of the scientific as well as general public. Crick wrote a letter to his son, sharing with him the news about the discovery. Sixty years later, in April 2013, this letter, known as Francis Crick's DNA letter sells at auction for a record $6 million. The letter was purchased by an anonymous buyer who made the bid over the phone. Half of the proceeds will go to Michael Crick and his wife. The other half will go to the Salk Institute for Biological Studies in California, where the elder Crick worked up until his death in 2004 at the age of 88 [12].

The importance of Chargaff's rules and diffraction photographs of DNA molecule in the discovery of the double helix

Combining of the bases according to the complementary base-pairing principle, suggested in Watson and Crick's model of double helix DNA, corresponded with Chargaff's findings about frequency of nucleotides in DNA molecule. As a result of his research, Erwin Chargaff (1905–2002) came to the conclusion that the content of purines in a DNA molecule equals the content of pyrimydines, and also, that the content of A equals that of T and content of G equals the content of C. In 1951, his results were mathematically formulated as $A/T = G/C = 1$ and $A + T = G + C$ and today these equations are known under the name the rules of Chargaff [13, 14]. Chargaff recalled the 3 lucky factors that contributed to his success: a new method described in 1944 by Consden, Gordon, and Martin that came to be known as paper chromatography – applied to the analysis of nucleic acid constituents, purines and pyrimidines; the availability for the first time of commercial ultraviolet spectrophotometers – which enabled the analytic procedure to be strictly quantitative – for purines and pyrimidines exhibit strong characteristic absorption spectra in ultraviolet; and what he felt to be most important, two excellent collaborators – Dr. Ernst Vischer and Mrs. Charlotte Green [15]. The scientific community of Serbia was introduced to Chargaff's work by the scientist himself who gave a lecture in the Serbian Academy of Sciences and Arts on June 22, 1970.

There is an anecdote about Chargaff who, while in London in 1952, had a meeting with Watson and Crick. They discussed the impact of his rules on the model of DNA structure. At this point Crick had forgotten the names of the bases, which did not impress Chargaff, who arrogantly considered that he was wasting his time talking to a couple of 'pitchmen' [16].

As already mentioned, radiography (X-ray) diffraction images of DNA were as important for the discovery of the secondary structure of this molecule as Chargaff's rules. Supportive of this opinion is the fact that the same volume of "Nature" in which Watson and Crick's article appeared also published two other articles confirming the suggested model with X-ray diffraction images of DNA molecule [17, 18]. The author of one of these two papers was Wilkins who later shared the Nobel Prize for Physiology or Medicine with Watson and Crick. The authors of the other were Rosalind Franklin (1920–1958) and Raymond Gosling (born 1926).

It is widely appreciated today that X-ray photographs made by Rosalind Franklin were of key importance for the discovery of the secondary structure of the DNA molecule [19]. In lecture notes dated November 1951, Franklin wrote the following: "The results suggest a helical structure (which must be very closely packed) containing 2, 3 or 4 co-axial nucleic acid chains *per* helical unit, and having the phosphate groups near the outside" [20]. However, because of her premature death at the age of 37, she could not be awarded the Nobel Prize since this prize cannot be posthumously awarded. In the speeches at the Nobel ceremony "Crick and Watson

notoriously and shamefully did not mention Rosalind, and Wilkins's tribute was slight, but who can say what might have happened if she had lived" [19]. Moreover, it is no secret presently that she did not know that they had seen either her X-ray photograph, showing unmistakable evidence of a helical structure, or her precise measurements of the unit cell (the smallest repeating unit) of the DNA crystal [21]. After she had done the work on diffraction images of DNA molecule and proteins, she left King's College and turned to the investigation of the tobacco mosaic virus structure. Rosalind Franklin published nearly 50 scientific papers. In the year she died her model of the tobacco mosaic virus was displayed at the international exhibition in Brussels, to be moved later to the new Laboratory of Molecular Biology in Cambridge [19]. Truth be told, during the last year of her life, she became a close friend of Crick and his wife Odile who she stayed with while receiving her treatments for ovarian cancer. After her death, Franklin has become a feminist icon – the Sylvia Plath of molecular biology – seen as a genius whose gifts were sacrificed to the greater glory of the male [21].

This is by no means the end of controversy regarding diffraction photographs of a DNA molecule. There can be no doubt Franklin's role was crucial: it was her skill in the technique known as X-ray crystallography that resulted in the famous Photograph 51. But it was Franklin's student, 22 year-old Raymond Gosling (Figure 2), who actually took the photo [17].

Fig. 2 – Raymond Gosling.

The anniversary itself was an opportunity for Gosling to recall the days he had spent in Rosalind Franklin's laboratory and of the Watson and Crick's discovery he designated as "Eureka moment" [22]. "Standing in the dark room outside this lead-lined room, and looking at the developer, and up through the developer tank swam this beautiful spotted photograph, you are familiar with them now I'm sure. It took 90-something hours to take the photograph, again, pot luck. But it really was the most wonderful thing. And I knew at the time that what I'd just done was to produce a crystalline state in these fibres, and if then the DNA was the gene material, I must be the first person ever to make genes crystallize" [23]. According to Gosling himself, the importance of Rudolf Signer (1903–1990), a Swiss biochemist should not be forgotten [23]**.** Rudolf Signer delivered a lecture in the Royal Society on his method for producing DNA of a superior quality. In Gosling's words: "Signer asked at the end of the lecture if anybody would like some of this material and he had a specimen tube full of this freeze dried material. Only two people put their hand up. I'm glad to say that Maurice (Wilkins) was awake enough to put his hand up!" [24]. Anyway, this scientific discovery, as any other, is a merit of many scientists whose work has been directed towards common goal, elucidation of the DNA secondary structure in this case.

The second half of the 20th century, a golden age of molecular biology

Shortly after the discovery of the secondary structure of DNA, the central dogma of molecular biology was postulated. It states that in the cell, the hereditary information is being transmitted in one direction from nucleic acids to proteins and never the other way around, and from DNA to DNA between generations. Quite a few scientists were involved in defining central dogma, and Crick was the most influential among them [25]. It is the opinion of many that defining this rule (in the year 1958) actually represents the beginning of the golden age of molecular biology.

Five years after the discovery of the DNA secondary structure, the American Matthew Meselson (born 1930) gave an experimental proof for the model of replication suggested by Watson and Crick [26]. During the mid 50s of the last century, American biochemist Arthur Kornberg (1918–2007) isolated the first enzyme involved in replication of DNA molecule. It was DNA polymerase I from *E. coli*. Using this newly discovered enzyme, Kornberg successfully synthesized DNA *in vitro* in the presence of deoxyribonucleoside triphosphate, molecules of DNA, magnesium ions and adenosine triphosphate. In addition, he discovered that this process always takes place in 5'→3' direction [27]. On the day Arthur Kornberg received the Nobel Prize, his wife, biochemist in the same scientific group, Sylvy Ruth Levy (birth: unknown-1986), gave a brief statement to the press: "I was robbed". The science is "family business" of Kornbergs. Apart from Kornberg and his wife, their two sons are also distinguished scientists. Roger David Kornberg (born 1947) is a professor of structural biology and one of the pioneers in the field of chromatin structure and function research. In 2006 he was awarded the Nobel Prize for his work on transcription in eukaryotic cells. His brother, Thomas Bill Kornberg (born 1948) was a member of research group which discovered enzymes DNA polymerase II and DNA polymerase III in 1970 [28, 29].

In the field of molecular biology, the beginning of the 60s of the last century was marked by the discovery of the role of messenger RNA (mRNA) in the flow of genetic information in cells and by introducing the notion of codon. In relation to this, Crick's adaptor hypothesis should be mentioned. In the year 1961, Crick and colleagues discovered that DNA "communicates in specific language" in which the words (codons) are always composed of three letters (each letter corresponds to one nucleobase of the DNA sequence). During these years, a great number of scientists were involved in the work on transcription and translation processes, but the discovery of mRNA is considered mostly the achievement of three of them: Matthew Meselson, Briton Sydney Brenner (born 1927) and French François Jacob (1920–2013) [30].

In 1963 the American Marshall Nirenberg (1927–2010) and Indian Har Gobind Khorana (1922–2011), based on the results of their independent research, explained the way in which nucleic acids (with 4 bases, that is 4 letters) determine the sequence of 20 amino acids in polypeptide chains using codons (three letter words). Nirenberg gave a correct estimate of the total number of codons – 64 ($4 \times 4 \times 4$). Through experiments started in 1961 in collaboration with the American Philip Leder (born 1934) he confirmed the hypothesis that nucleic acids determine the sequence of 20 amino acids using 64 codons [31, 32].

The year 1974 was marked by one of the most unexpected discoveries in the molecular biology which had a tremendous influence on its further development. The Americans Richard John Roberts (born 1943) and Phillip Allen Sharp (born 1944) discovered that eukaryotic genes consist of coding (exons) and non-coding (introns) sequences. After transcription, the processing of the primary transcript takes place, which includes splicing out introns and joining together of exons to form the strand of mRNA [33].

The first recombinant DNA molecule was created in the begging of 70s of the 20th century, which initiated the new era of recombinant DNA technology. American biochemist Paul Berg (born 1926) created *in vitro* the first hybrid circular DNA molecule using the sequences of viral (*SV40*) and bacterial (*E. coli)* DNA [34]. This was possible only due to the discovery of restriction endonucleases and DNA ligase. The first isolated restriction enzyme was *Hind* III from the bacteria *Haemophilus influenzae*. It was isolated in 1970 by three microbiologists – the American Hamilton Smith (born 1931), the Swiss Werner Arber (born 1929) and another American Daniel Nathans (1928–1999) [30].

During the mid-seventies of the last century first techniques for DNA sequencing were developed. The British Frederick Sanger (born 1918) and the American Walter Gilbert (born 1932) independently created original methods to determine the sequence of nucleotides in DNA molecule [35, 36]. By applying his own method, Sanger managed to sequence a stretch of the DNA from *E. coli* bacteriophage ØX174 whose genome is 5.375 base pairs (bp) long [37]. Frederick Sanger, double laureate of the Nobel Prize, is one of the most famous living scientists.

Entry into the genomic era

With the development and automation of methods for DNA sequencing it was possible to consider sequencing of the human genome and genomes of other species. The idea about deciphering the human genome originated in the US Department for Energy in 1985. This idea was met with the approval of the scientific community and as a result, in 1988, the National Center for Human Genome Research (NCHGR) was founded. The estimate of the number of genes in human genome revolved around 100,000 at the end of the 80s of the last century. Based on this estimate the plan was devised for scientists from NCHGR to sequence the entire DNA in human cells over period of 15 years spending the budget of 3 billion dollars. October 1990 is usually considered as the official launch date of the project. At the very start it was clear that it surpasses the scope of a national project. It became an international project as the scientific institutes from the European Union, China, Japan, Australia and other countries (18 in total) joined. The Human Genome Organization (HUGO) was founded first with the task to coordinate the work of this large number of institutions and later the International Human Genome Sequencing Consortium with the same purpose. Watson was the director of the consortium for a period. One of the founders and directors of the NCHRG was Craig Venter (born 1946). Due to the misunderstanding regarding the methodology of sequencing, Venter leaves NCHGR and founds his private institute – TIGR (the Institute for Genomic Research) [38]. This institute succeeds to sequence the first whole genome of an organism in less than six months. It was the genome of the bacterium *Haemophilus influenzae* 1.830.137 bp long and consisting of 1,749 genes [39]. After deciphering the first genome of a living organism, interest in deciphering the human genome was growing over years. The number of pharmaceutical companies and companies of other type that would readily make considerable investments in this project was also growing. In 1998 Venter starts the private company "Celera Genomics". After the sequence of the first prokaryotic genome had been published, one of the most exciting races in the history of science begun. Its participants were scientists from NCHGR and from "Celera", while its goal was to read out the complete sequence of the human genome [38].

As the work progressed towards the end, the rivalry was becoming more open. Arguments in favour of one or the other sequencing strategy were the topic of many scientific, but also social debates. The confrontations of the two teams with two different approaches and mutual challenges threatened the whole project. Under the circumstances the US president Bill Clinton was bound to intervene. He organized meeting of the leaders of both teams in the East Room of the White House on June 26, 2000. Presidential mediation led the scientists to bury the hatchet and shake hands [40]. The wish to successfully complete the project of deciphering the human genome overtook. The exchange of the results of readout of the human genome obtained by that moment was arranged between the teams. The result of described efforts

was a successful completion of one of the most expensive scientific projects of all times – the deciphering of the human genome.

Ending of a project of this sort is the culmination of the investigations that started with the discovery of the secondary structure of the DNA molecule. At the same time it is the starting point for the new scientific branch named genomics. The research in genomics extends over many disciplines mostly including: sequencing of the genomes, determining the number and the structure of the genes, investigations of the gene expression profiles and determining the structure and function of proteins. Comparative genomics has as its main topic investigations into similarities and differences between the genomes of different species. In parallel with genomics another novel scientific discipline develops – bioinformatics. Bioinformatics is defined as application of information technologies in biology. It refers primarily to the collection, processing and analysis of experimental results. Deciphering of the billions of base pairs of the hundreds of genomes of different species is currently underway. The work of such a large scale requires the creation of specific data bases. The final goal of this approach is to make results of the work of scientists investigating genomes of many organisms accessible *via* Internet to all interested researchers around the world. The future of genomics can be seen in the application of its findings in numerous sciences such as agronomy (agrogenomics), pharmacology (pharmacogenomics), and especially medicine (genomic medicine) [41].

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