Paul Berg and the Dawn of Genetic Engineering

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Abstract- Paul Berg is one of the most important and innovative scientists of the 20th century. He was a pioneer in the use of advanced techniques for handling nucleic acids that caused a revolution in the medical sciences, agriculture and industry related to Biotechnology. Genetic engineering allows the manipulation, structural alteration and cloning of genes, in addition to fragments of DNA and RNA that also carry regulatory sequences of gene expression. The effects and consequences of the application of genetic engineering to people's daily lives are immeasurable and represent a great improvement in the quality of life of patients, in human nutrition and in the production of safer and better quality manufactured goods.

Keywords – Paul Berg, genetic engineering, gene cloning, Biotechnology

I. INTRODUCTION

The simplest and most popular definition for Biotechnology is that it is an area of applied Biology related to the use of living organisms to obtain a useful biological product or process for humans.

A more complete and modern definition conceptualizes Biotechnology as the wide range of techniques and actions for manipulating living beings, or parts of living beings to obtain functional products through microbial fermentations, the techniques of plant and animal tissue culture and genetic engineering.

In this context, the role of genetic engineering can be defined as the use of biological methods, recurrent to certain microorganisms, to modify the genetic material of living cells. It is necessary to understand under what circumstances the idea of genetic manipulation advocated by genetic engineering developed. It is inevitable not to associate the development of genetic engineering with that of Genetics itself, since the first is also called recombinant DNA technology, gene manipulation, gene cloning and also the "New Genetics".

Genetics began properly from the rediscovery of Gregor Mendel's works with crosses and hybridizations with pea plants, at the turn of the 19th to the 20th century, which resulted in the elucidation of the principles that govern the inheritance of characters from generation to generation.

An earlier event, however, was already beginning to fuel interest in inheritance mechanisms at the molecular level. From the first isolation of a complex molecule of undefined structure, characterized as nuclein, later called DNA deoxyribonucleic acid, carried out in 1869 by the Swiss biochemist Friedrich Miescher, a new “era” began in Natural Sciences.

Back in the early 1940s, the work of microorganism geneticists and microbiologists boosted understanding about the transfer of genes between bacteria and generated a wide base of foundations for scientific development in this area to come (Nicholls, 2001). Through the experiments conducted by Oswald Avery, in 1944, aiming at understanding the functioning of penumonia caused by bacteria, evidence was provided that deoxyribonucleic acid is the carrier molecule of genetic information, linking it directly to the inheritance mechanism (one more molecular level clue to character inheritance).

Certainly, the greatest contribution and stimulus to the development of molecular genetics and, later, genetic engineering, was given in 1953, with the proposition by James Watson and Francis Crick of the double-helix model for the structure of DNA - based on the results of X-ray diffraction by Rosalind Franklin and Maurice Wilkins. At that time, the foundations were established to understand the way in which genetic information is stored and how it is structured.

This, which is perhaps the greatest scientific landmark of the 20th century, boosted scientific activity in this field in an impressive way, characterizing the 1950s and 1960s as the golden twenty years of molecular genetics. From the second half of the 1950s, numerous discoveries at the molecular level provided new paths for understanding and for the in vitro replication of the manipulation of the DNA molecule, which would only be carried out successfully in the early 1970s.

The combined action of the discovery of DNA polymerase by Kornberg in 1957, the renaturation of the two strands of DNA by Marmur and Doty in 1961, the first evidence of the existence of DNA restriction endonucleases (and their subsequent purification) proposed by Arder in 1962, the characterization of DNA sequences by Nathans and H.Smith also in 1962 and, finally, the discovery of DNA ligase in 1967 by Gellert, allowed to obtain the concepts that would lead to the development of DNA cloning techniques proposed by Berg et al., 1973, resulting in the controlled alteration of the molecule of life. It was the beginning of the era of genetic manipulation.

The character and persistence of researchers and research groups spread across western Europe, the Soviet Union, Japan and other countries where research was carried out at the molecular level must be highlighted, since in the early 60s, in the middle of the road, a feeling fructification has spread within the scientific community. Molecular geneticists found themselves at a crossroads generated by technical limitations, in terms of rudimentary equipment and insufficient laboratory tools, which hampered progress in the area. Solutions to these problems were generated through a lot of imagination, study and public and private investments aimed at improving the technological apparatus of the time.

In this scenario, according to Nicholl (2001), the development of genetics can be divided into three large periods of different origins but overlapping at certain times: Classical Mendelian Genetics, Microbial Genetics and Genetic Engineering.

The progressive gathering of information provided by the discoveries of the 1960s was decisive for the development of the process of maturing in genetic engineering, but they would be insufficient if they were only "fit" like pieces of a puzzle.

The genius of Paul Berg, one of the fathers of genetic engineering, is verified precisely because he did not simply group the foundations and ideas obtained by the discoveries of his predecessors. Its proposal for the isolation of a DNA fragment from an initial cell, its integration into a bacterial nucleic acid vector (called a plasmid) and its subsequent introduction and integration into the genome of a target cell was extremely advanced and avant-garde.

The idea that a fragment of DNA introduced into a cell different from that of its origin, inserted in a biochemical and biophysical environment completely different from the native, could be integrated in a stable way into the genome of the recipient cell and, more unlikely, have its functional character verified experimentally it was seen as something of very low probability.

Paul Berg was born in Brooklin, New York, on June 30, 1926, to a middle-class family. His interest in science began by contacting two books in his teens: Arrowsmith, by Sinclair Lewis and Microbe Hunters, by Paul de Kruif. Berg was admittedly an exceptional student, having graduated in biochemistry in 1948 from Pennsylvania State University.

After serving in the navy from 1944 to 1946, he was accepted at Western Reserve University, where he obtained a Ph.D. degree in biochemistry, in 1952.Seo postdoctoral research was fundamental to the direction his scientific life would take years later, since he had the privilege of being a disciple, from 1952 to 1953, of Herman Kalckar, one of the greatest cytologists of his time, at the University of Copenhagen, Denmark, and also of Arthur Kornberg, at Washington University, in the 1953/1954 biennium.

Kornberg had an enormous influence on the way Berg came to view molecular mechanisms at the enzymatic and macromolecule levels such as DNA. According to Berg himself, Kornberg was: "an inspirational leader on the frontier of molecular and cellular biology".

His research at Washington University involved converting fatty acids into their active forms, leading him to co-discover aminoacyl tRNA synthetases and tRNAs. In 1959, Berg moved to Stanford University in California, becoming an associate professor of biochemistry at the School of Medicine. His investigative work on identifying the stages in which DNA makes proteins led him to successfully splicing (fragmenting and rearranging) genetic material from one living organism to another, in 1973, for which he won the Nobel Prize for 1980 Chemistry.

In this work, Berg developed a methodology to introduce new genetic information, derived from the circular DNA of the bacterium Escherichia coli, into the genome of the SV40 virus, a pathogen of simians.

According to Berg: “the recombinant viral DNA λ-SV40 produced by genetic manipulation experiments contains the biological information to encode almost all the functions of the original SV 40 DNA and all the functions of the *E.coli* galactose operon, as well as the information of λ bacteriophage required for the autonomous replication of circular DNA molecules in E.coli. ” This means that the bacterial DNA fragment cloned by Berg in the virus genome, proved to be functional and perfectly active.

In general, genetic engineering allows for in vitro manipulation and cloning of genes, resulting in obtaining complete or fragmented bacterial plasmid vectors. These hybrid molecules contain sequences coding for RNAs - which may or may not be translated into proteins - associated with the genetic elements that control gene expression, such as promoters, signal peptides and terminators. The set of genetic elements that form the exogenous unit of gene expression constitutes the expression cassette, which can be stably integrated into the host genome.

Gene cloning, as the technology developed by Berg was initially known, represented a real revolution in molecular biology, since it allowed the expression of exogenous genes in receptor cells different from those of their origin and, in a more comprehensive view, the study of cells and their macromolecules much more deeply. Now, it was even possible that beings from different evolutionary domains and kingdoms could share hybrid and functional fragments of DNA. The barriers to evolutionary compatibility had been circumvented ingeniously.

Some tangible examples of this revolution could be seen in the prospecting of genes and in the discovery of new classes of genes and proteins. Very informative studies about the evolution of macromolecules, obtaining new tools for the determination of gene and protein functions, the characterization of regulatory sequences of genes and, consequently, the elucidation of the mechanisms that regulate gene expression in eukaryotes, made possible mass production recombinant proteins such as human insulin and commercial vaccines, among numerous other important applications (Alberts, 1994).

Forensic genetics and paternity testing have been improved. Now, it was possible to obtain sufficient amounts of DNA to carry out tests even in material scarcity scenarios.

DNA sequencing based on the shotgun technique, with the cloning of genomic fragments, amplification in bacteria or yeast and assembly of contigs was also a milestone in genetic engineering.

Perhaps, the field that has benefited most from gene cloning was agriculture. The use of genetic engineering as a tool for assisted plant breeding was initially aimed at improving agronomic characteristics, such as resistance to diseases and pests, tolerance to different forms of biotic and abiotic stresses, obtaining a longer shelf life for fruits, the promotion of changes in the color patterns of ornamental flowers and the achievement of male sterility (Daniell, 1999).

Since the first transgenic plant was produced by researchers at the University of Washington (USA), in association with the University of Gent (Belgium) and the American company Monsanto in 1983 - a tobacco plant containing a bacterial kanamycin resistance gene - efforts of numerous researchers in the most varied centers of scientific investigation have been directed to potentiate the use of superior plants as vehicles of expression of a huge range of heterologous proteins, with the most diverse biological functions (Kusnadi et al., 1997).

Berg was invited to join the National Academy of Sciences in 1974, and expressed concern about the use of genetic engineering irresponsibly or without strict criteria of bioethics. Thus, Berg was a prominent figure at the Asilomar Conference, in 1975, an event that discussed potential risks of applying recombinant DNA technology and established the regulatory limits for genetic manipulation.

His research in genetic engineering continued until 1991, when he was appointed adviser to the Science Advisory Committee of the Human Genome Project. In 2002, forty Nobel winners, including Berg, spoke out in favor of nuclear transfer therapy aiming at the production of stem cells for therapeutic research purposes, with the hope of finding a cure for diseases considered to be of greatest weakness in human health. .

Currently Paul Berg, aged 94, remains attached to Stanford University, where he is professor emeritus at the Cancer Research Institute and director emeritus at the Beckman Center of Molecular and Genetic Medicine.

II.CONCLUSION

As a result of the efforts of more than 40 years of work on molecular genetics, three milestones of the achievements of genetic engineering were reached in the 1980s: the production of transgenic mice by Palmiter and Brinster in 1981, of transgenic fruit flies in 1982 by Spradling and Rubin and the development of the DNA fragment amplification technique by polymerase chain reaction, in 1984, by Mullis and his collaborators (Alberts, 1994).

All of these molecular techniques, despite being relatively new, represent a true revolution in cell biology, since they allowed the expression of exogenous genes in receptor cells different from those of their origin and, in a more comprehensive view, the study of cells and of its macromolecules in more depth.

Some tangible examples of this revolution can be seen in the prospecting of genes and in the discovery of new classes of genes and proteins.

Very informative studies about the evolution of macromolecules, obtaining new tools for the determination of gene and protein functions, the characterization of regulatory sequences of genes and, consequently, the elucidation of the mechanisms that regulate gene expression in eukaryotes, made possible mass production recombinant proteins such as human insulin and commercial vaccines, among numerous other important applications (Alberts, 1994).

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