

## **Fifty years after Watson and Crick**

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### **1. Introduction**

The discovery of the molecular structure of DNA by Watson and Crick in 1953 can be considered to be equivalent to the discovery of the wheel. The impact of these discoveries on mankind have been so profound that it is difficult to imagine what the world would be today without them. The recognition of the potential of the work of Watson and Crick which was published in April 1953 in the Nature Magazine<sup>1</sup> was such that they were awarded the Nobel prize for physiology and medicine in medicine in 1954.

Looking back and tracing the developments stemming from this discovery, there is no difficulty in recognizing that Watson and Crick had indeed laid the foundation stone for the development of the branches of science now known as molecular biology and genetic engineering. An attempt would be made in this article to outline the profound impact the revelation of the structure of DNA had made on science and mankind within the last 50 years.

#### **Identification of the genetic material.**

From perhaps the time man acquired the ability to rationally think and observe, he was aware that the offspring of living organisms were a combination of their parents characteristics. In other words man had observed heredity. Then in 1866 Gregor Mendel came along and transformed this awareness into a science through his now famous work on garden peas. By 1902 Theodor Boveri and Walter Sutton working independently established that this heredity was brought about by the action of genes located on the chromosomes<sup>2</sup>. The answer to the question what genes are made up of, took a while to come. Even though nucleic acids were

identified way back in 1869 by the Swiss chemist Friedrich Miescher, it was only in 1944 that O.T. Avery, Maclyn McCarty and Collin Maleod showed that DNA is the genetic material by establishing that only DNA is capable of transforming bacteria<sup>3</sup>. Finally, in 1952, Alfred D. Hershey and Martha Chase working with the bacteriophage T2 established that DNA alone is the genetic material.

### **Molecular structure of the genetic material.**

The grand finale to the story of the genetic material was provided by Watson and Crick in 1953 when they revealed the molecular structure of DNA (figure 1). Their work brought them the Nobel Prize in 1954, just about a year after the publication of the now famous article in the Nature magazine. The proposed structure elegantly explained how the DNA could perform all its known functions. This discovery not only laid the foundation for the establishment of the new branches of Biochemistry, namely Molecular Biology and Genetic Engineering, but also stimulated a “big bang” in research activities in biochemistry resulting in astounding developments making a profound impact on humanity through agriculture and medicine.

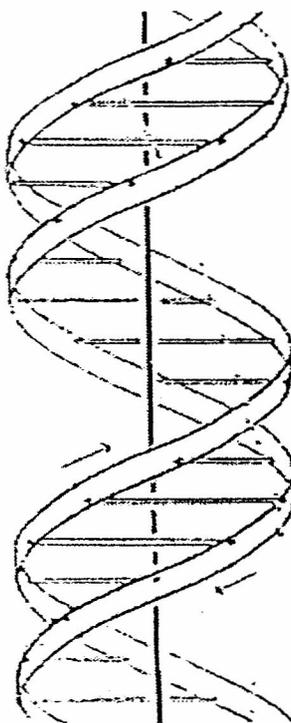


Fig 1. Diagrammatic molecular model of Watson and Crick as published in Nature magazine in 1953.

It is beyond the scope of this article to go into the details of the molecular architecture of DNA proposed Watson and Crick<sup>4</sup>. In summary, Watson and Crick proposed that the DNA was a by double helix resembling a twisted ladder with two sugar phosphate backbones making up the sides with the steps of the ladder being made up of the four bases (Adenine, Thymine, Cytosine, Guanine) hydrogen bonded in a complementary manner, with the purine base bonded to the pyrimidine base. By this complimentarily they meant that Adenine (A) is always hydrogen bonded to Thymine (T) while Cytosine (C) is always bonded to Guanine (G). Thus the only possible variable in the proposed molecular structure of DNA was in the arrangement of the four bases along the molecule.

With the realization of this complimentary of base pairing, the mechanism of the accurate duplication of DNA at cell division could be envisaged. Then, in 1961, less than ten years after the work of Watson and Crick, Marshall Nirenberg and Heinrich Matthei laid the ground work for the deciphering the nature of the genetic code. In their classic experiment using a synthetic poly "U" RNA and a cell free extract of *E. coli* they demonstrated that only a polypeptide of phenylalanine was formed prompting them to suggest that the "triplet" code of UUU coded for phenylalanine. Then in 1964, Marshall Nirenberg and Philip Leder demonstrated that poly U mRNA will bind only phenylalanine-tRNA and not any other amino acyl tRNA. At about this time complementary research by Gobind Khorana established methods of synthesising polyribonucleotides with defined repeating sequences of bases. By 1966 the combined work of Marshall Nirenberg and Philip Leder as well as Gobind Khorana and other researches, the entire genetic code was deciphered. Soon the process of transcription and translation were established and developments proceeded at an unprecedented rate leading to the birth of Biotechnology or DNA based technology.

### **The birth of Genetic Engineering.**

While all this was going on another major development took place in late 1960s when Werner Arber and Stewart Linn discovered the presence of a nuclease that broke down DNA in *E. coli*. These enzymes came to be called restriction endonucleases. However these nucleases were all unspecific because they cleaved the DNA at random locations. Then in 1970 Hamilton Smith of Johns Hopkins University isolated the first specific restriction endonuclease. These enzymes cuts through both strands of long DNA molecules at specific sequences within the molecules resulting in smaller fragments. Further, the cut through these specific sequences, called palindromes, are frequently staggered creating sticky ends because of the complementary base pairing. Thus DNA from different sources cut with the same restriction enzyme could join up by complementary base pairing on account of the sticky ends resulting in a "mixed DNA" molecule now referred to as a recombinant DNA.

The 1970s, just about twenty years after the landmark work by Watson and Crick, turned out to be eventful. Just about three years after the isolation of specific endonucleases, in 1973, Stanley Cohen and Herbert Boyer created the first recombinant DNA in a test tube. Using the restriction enzyme *EcoRI* and two different plasmids pSC 101, which carried the tetracycline resistant gene, and the RSF 1010 plasmid which carried the streptomycin resistance gene, Cohen and Boyer created a recombinant plasmid in a test tube which carried both the streptomycin and tetracycline genes. Thus “recombinant DNA technology”, or “Genetic engineering” which enables genes to be removed from one organism and inserted into another organism was born. This technique along with the technique of cloning a recombinant DNA and other related techniques gave humans the unprecedented opportunities of manipulating the genomes of organisms for his benefit. Listing the complete set of biotechnology products available to date is beyond the scope of this article. As such only some products and techniques of technology and their applications in medicine that are particularly useful to humans will be dealt with in this article.

#### **Some applications of biotechnology.**

The ability to produce proteins through genetic engineering techniques opened up a new frontier through the production of proteins of pharmaceutical value. The first cloned protein of pharmaceutical value that was made available was insulin. This was achieved by cloning the human insulin gene in a bacterium and inducing the bacterium to produce the human insulin outside the human body. Before the advent of biotechnology, insulin for diabetics was either porcine or bovine insulin extracted from animal pancreatic tissue. Thus biotechnology assured the supply of human insulin without having to depend on the supply of livestock. More importantly, the human insulin from the cloned gene was much safer because the insulin was indeed the human protein and hence had only a minimal chance of triggering an immune or allergic response in the patients. A second such protein is the human growth hormone, the deficiency of which causes hypopituitary dwarfism, an inherited disease. Children affected with this disease were formally treated with growth hormone extracted from pituitary glands of human cadavers with an accompanying risk of precipitating a lethal brain condition. The treatment was very expensive because one year requirement of the hormone for the treatment of one patient required more than seventy cadavers. Cloning of the human growth hormone gene and expressing it in micro organisms provided acceptable solutions for these problems.

Genetic engineering paved the way for the synthesis of other proteins of pharmaceutical value such as clotting factors for patients affected with haemophilia as well as genetically engineered urease for accelerating the removal of urea from the blood in kidney failure. Monoclonal antibody technology for the production of useful antibodies is another new area that arose from the developments of genetic engineering

Yet another contribution of Recombinant DNA technology by producing vaccines from cloned genes for the alleviation of human suffering. A good example is the hepatitis B vaccine<sup>5</sup>. Vaccines produced through classical procedures had certain disadvantages such as the vaccine itself precipitating the disease. The genetically engineered vaccines surmounted most of these disadvantages making the vaccine much more safer.

The pinnacle of achievement in genetic engineering appears to be its application in gene therapy even though the technology is still in its infancy. The goal of gene therapy is to insert a cloned normal gene into somatic cells carrying a mutated defective gene. This strategy has been used in the treatment of patients with severe combine immunodeficiency disease. However there are risks of this treatment as the inclusion of the normal gene has led to the activation of a hematopoietic oncogene as a few patients developed leukaemia<sup>6</sup>.

While on the topic of oncogenes, it would be a lapse to omit mentioning that the recognition of these genes that cause malignancies would not have been possible if not for the ground breaking work of Watson and Crick. Much is known now regarding the role played by oncogenes in switching a normal cell to a malignant cell.

Apart from these, DNA based technology has contributed immensely in diagnostics including prenatal<sup>8</sup>. Restriction Fragment Length Polymorphisms (RFLPs) have been useful in the prenatal diagnosis of the dreaded X chromosome linked disease Duchanne muscular dystrophy<sup>9,10</sup>. This technology has opened new frontiers in forensic science facilitating law authorities track down criminals with the help of variable number tandem repeats (VNTRs) analysis of tiny biological samples left behind scenes of crime. This is what is now referred to as DNA fingerprinting.

Just 50 years down the line since the landmark paper by Watson and Crick, the technology for creating transgenic animals and plants are here to stay. Scientists have now incorporated foreign genes into animals (mammals) and plants (Angiosperms) to incorporate desired characteristics.

Way back in 1983 Richard Palmiter and Ralph Brinster placed the human gene for growth hormone in a mouse embryo and produced a mouse with double the normal size. This was a "transgenic" animal. The technology has since been extended to use animals such as goats to produce important proteins by introducing the respective human gene into them<sup>2</sup>. For instance human antithrombi III is being secreted in the milk by transgenic goats.

Transgenic animals will undoubtedly play an important role in livestock farming in the future by having a higher growth rate, by being bigger and more resistant to disease. For instance transgenic pigs with bovine growth hormone has been shown a greater feed efficiency, ie weight gain per unit of feed.

In the field of agriculture too the developments are remarkable with the production of transgenic plants with desirable characters from a human point of view. The ability to create transgenic plants raises the possibility of obtaining new strains of crop plants that are superior with respect to yield, drought resistance, insecticide and herbicide resistance as well as quality of proteins produced.

One of the first herbicide resistant plants were developed by David Stalker and his co-workers when they engineered a glyphosate resistant tobacco plant. Glyphosate is a herbicide which inhibit the enzyme EPSP synthase which is required for the synthesis of essential amino acids phenylalanine, tryptophan and tyrosine by plants. Thus it is not possible to apply glyphosate to a field because the herbs as well as the crop plants would be killed. David Stalker and co-workers inserted a mutant EPSP synthase gene which coded for a glyphosate resistant enzyme into tobacco plants resulting in tobacco plants resistant to glyphosate. Much work is now under way to engineer desirable crop plants for meeting the demands of the human race.

No account of transgenic plants would be complete without mention of the transgenic tobacco plant carrying the luciferase gene of fireflies. This transgenic tobacco plant glows in the night !! Much use of such an exercise is difficult to foresee at the moment, but you never know what the future will hold. Such plants might replace street lamps some day in the future, at least to some extent.

### **Some applications of stem cell technology**

In 1998, the isolation of human embryonic stem cells by two research groups in the USA opened up remarkable new opportunities for the treatment of a wide range of degenerative diseases. These may enable the creation of replacement cells to treat disease conditions such as Parkinson's disease, heart muscle failure and diabetes.

The ability to grow a wide variety of human tissues opens the door for treating a range of cell based diseases and growing medically important tissues that can be used for transplantation processes, such as for juvenile onset of diabetes mellitus and Parkinson's disease occur due to defects in one of just a few cells type, replacing faulty cells with healthy ones offers hope of life long treatment. Similarly, failing hearts and other organs, in theory, could be shared up by injecting cells to replace damaged or diseased cells. However, in general it has proven very difficult to isolate and propagate adult stem cells as they are already specialized and their potential to regenerate damaged tissue is very limited. Therefore only embryonic stem cells have a potential capacity to become any kind of human tissue to repair vital organs<sup>11,12</sup>. Stem cell technology, therefore would permit the rapid screening of hundreds of thousands of chemicals for discovery of novel drugs that must now be tested through much more time consuming processes.

Having realized the importance of biotechnology in the development of science and technology for developing countries it is timely and noteworthy to mention the progressive steps taken in Sri Lanka to establish an “Institute of Molecular Biology and Biotechnology” by the University of Colombo in May 2004 which coincided with the dawning of fifty years after Watson and Crick.

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