World Journal of Medical Sciences 2 (2): 65-77, 2007
ISSN 1817-3055

Genetics and Biotechnology in Historical Perspective: A Review

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Abstract: Our interest in heredity and transmission of traits from one generation to next can be traced back to at least 10,000 years. Despite the impressive historical record, the nature and conception of heredity largely remained speculative until recent times. Throughout the history, genetics had a profound effect on humankind and the general principles of heredity as discovered by Mendel in green peas are still applicable to all the living organisms. Genetics today largely is the result of research that was performed during the 20th century. Although DNA was discovered in 1869, discovery of physical structure of the miracle molecule of life in 1953 by Watson and Crick marked the beginning of modern genetics. As a result of research in genetics and advances in the field of biotechnology, the major benefits have been in the areas of agriculture and medicine. Recombinant DNA technology has produced fundamental changes in the diagnosis and treatment of many genetic disorders. Human genome project (HGP) that was completed in 2003 has contributed immensely to our understanding that how gene defects can cause disease. Bioinformatics and proteomics are new emerging fields. It is hopped that future research in proteomics will shed some information that how bacterial proteomates change with the alteration in the environment. With the recent set backs in the gene therapy trials,attention is currently focused on embryonic stem (ES) cell technology. The scientists believe that ES cells could be one of the greatest revolutions in modern medicine curing an array of diseases form Alzheimer to spinal cord injury. The use of small interference RNA (siRNA) as therapeutic tool and its potential use to block the disease process is focus of current research in medical genetics.

Key words: Biotechnology · medical genetics · stem cell technology · molecular biology

INTRODUCTION

Genetics is the study of inherited traits and their variations. The inheritances of physical traits have been a subject of curiosity and interest for thousands of years. We are not sure when the people first recognized the existence of heredity, but the prehistoric (between 8000-1000 BC) evidences of cultivated plants and domesticated animals clearly document our ancestors successful attempts to manipulate the genetic composition of useful species. There is little doubt that ancient people soon learned that the desirable and undesirable traits can be passed from one generation to the next and more desirable varieties of animals and plants could be bred. Human awareness of heredity was thus apparent during the prehistoric period. Few significant ideas were put forward by ancient Greeks and other medieval scholars to explain heredity during the prehistoric times; but these were related to the origin of humans and to reproduction and hereditary in particular.

Significant progress was made between 1600-1850 AD that provided a greater insight into the biological basis of life, paving the way for the revolutionary work and principles presented by Charles Darwin and Gregor Mendel [1]. In early 1600's William Harvey put forward the theory of *Epegenesis* suggesting that an organism desired from a substance is present in an egg differentiates into adult structure during the embryonic development. About 1830, Mathias Schleiden proposed the cell theory that all organisms are composed of basic visible units called cells. The work of Charles Darwin on evolutionary theory *"the Origin of Species"* published in 1859 convinced him that the existence species arose by descent formulation from other ancestral species [2]. This thinking culminated in the formulation of the theory of natural selection, a theory that attempted to explain the cause of evolutionary changes. Gregor Mendel advanced the field significantly by performing a series of experimental designed on garden peas. He used this experimental information to formulate a series of

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fundamental principles of heredity [3] suggesting that traits were transmitted from parents to progeny by discrete dependent units later called *"Genes"*. Mendel further proposed hat a gene (a unit of heredity) can exist in two different forms (dominant and recessive) and each parent carried two copies of a gene. Homozygous was those who carried two copies of the same allele (an alternative form of gene) and the heterozygous with one copy of the allele. Consequently the terms *"genotype and phenotype"* were introduced.

It is fair to say that the field of genetics has arisen form agriculture. Traditional agriculture was used for controlled breeding of plants and animals to select individuals with certain dominant traits. Biotechnology is commercial and industrial processes that utilize biological living organisms or products. There are several examples of ancient biotechnology e.g., micro-organisms were used to ferment fruits to manufacture alcoholic beverages by Babylonians in 6000 BC. Yeast was used to bake bread by Egyptians around 4000 BC and Chinese used chrysanthemum as an insecticide in 100 AD (Appendix I). Vaccine technology dates back to eleventh century in China where people collected the scabs of individuals infected with smallpox and crushed them into powder, where they inhaled or rubbed into pricked skin.

In 1970 Hamilton Smith [5] described the isolation of a restriction enzyme from a bacterial strain that can cleave the viral DNA at specific sites. This enabled researchers to manipulate one gene at a time and create hosts that can harbor new genes or that over- or under-expressed their own genes. This revolutionized almost all fields of experimental molecular biology and became the foundation of biotechnology and creating the science of genetics engineering. The resulting organisms are now technically termed genetically modified (GM), or more specifically, an organism with genes from another species is termed *transgenic*. Golden rice for example manufactures beta-carotene (a vitamin A precursor) using transgenes from petunia and bacteria. It has twice the iron compared to unaltered rice because one of its own genes is over-expressed [6].

Biotechnology is now an emerging field in food and its specific applications in food biotechnology, human health and diagnosis, industry and environment are few to mention. There were several agricultural challenges on which the scientists worked deliberately and as such agriculture have been improved in resistance to disease and insect and hybrid varieties have desirable qualities such as increased protein values. Over the past four decades genetic manipulations have produced many transgenic plants and GM crops have revolutionized the agriculture, however much of the concern centers on issue of safety [12]. In general, if proteins are neither toxic nor allergenic and do not have any other negative physiological effects, they are not considered to be a hazard to health. In 1990's EPA approved that the genetically engineered foods were "not inherently dangerous" and did not require special regulation. In 1983, the U.S. patents were granted to companies involved in genetically engineered plants. This was a big boost and as such the United States itself now accounts for two-third of world production of genetically modified crops (soybeans, corn, cotton, tobacco and others), Canada (7% canola) and China (1% cotton). Products of recombinant technology are also used in the food industry e.g. enzyme rennin that is normally produced in calves is now being produced genetically. The gene that encodes the enzymes is inserted into a plasmid and transformed into bacteria, which are mass cultured to produce large quantities of pure rennin.

The contributions of biotechnology in medicine are also unrivaled. Insulin, the first human gene product was manufactured by using recombinant DNA [13]. Genetech was the world's first genetic engineering company and in 1982, it received the FDA approval to market genetically engineered human insulin. We have now synthetic human insulin that is produced by another method, in which synthetic nucleotides encoding the insulin A and B chains are inserted at polylinker site of a cloned *E. coli* P-galactosidase gene. The recombinant plasmids are transformed into *E. coli,* where the P-gal /insulin fusion proteins is activated and synthesized in the host cells. Fusion peptides (proteins) are then extracted from the host cells and purified. Insulin chains are then released from p-galactosidase with cynogen bromide. The insulin subunits are purified and mixed to produce a functional insulin molecule. Several genetically engineered proteins (Table I) for therapeutic uses have been produced by similar methods. This involves the cloning of a human gene into a plasmid and inserting the recombinant vector into bacterial host producing a protein. After ensuring the transformed gene is expressed, large quantities are produced and human proteins are recovered and purified.

One of the most useful applications of biotechnology is the production of vaccines that can stimulate an immune system response to produce antibodies against a disease. Two types of vaccines commonly used are, inactivated (prepared from killed samples of infectious virus or bacteria) and attenuated (live viruses or bacteria that can no longer reproduce but may cause a mild

Table 1: Genetically Engineered Pharmaceutical Products

Drug	Condition Treated
Atrial natriuremic factor	Blood vessel dilation
Colony stimulating factor	Cancer chemotherapy; bone marrow-transplant
Deoxyribonuclease (Dnase)	Cystic fibrosis
Epidermal growth factor	Burns; skin transplantation; improves healing
Erythropoietin (EPO)	Anemias
Factor VIII	Hemophila
Fertility hormones (FSH,LH,HCG)	Infertility
Glucocerebrodiase	Gaucher disease
Granulocyte colony stimulating factor	Cancer
Hepatitis B vaccine	Hepatitis
Human growth factor	Dwarfism
Insulin	Diabetes mellitus
Interferons	
Aplha	Hairy cell leukemia; Hepatitis C; Kaposi sarcoma
Beta	Multiple sclerosis
Gamma	Chronic granulomatous disease
Interleukon-2	Cancer
Lung surfactant protein	Respirartory distress syndrome
Renin inhibitor	Blood pressure
Superoxide dismutase	Transplant; prevents damage to heart muscle
Tissue plasminogen activator	Heart attack

disease). There are now several genetically engineered vaccines that are commercially available for many bacterial and viral diseases. Biotechnology is also being used to produce a new type of vaccine called, subunit vaccine that consist of one or more surface proteins of virus or bacterium [14]. One of the first subunit vaccines was for hepatitis B, a virus that causes liver damage or cancer.

The application of DNA polymorphism has also revolutionized the forensic medicine. Alec Jeffreys who coined the term DNA fingerprinting discovered multilocus probes in 1984. He was the first to use DNA polymorphism in paternity, immigration and murder cases. These probes arose from the investigations of hypervariable regions composed of short repeated sequences of DNA, minisattelites or cluster of 10-100 nucleotides [15]. Clusters of such sequences are widely dispersed in the human genome and the number of the repeats at each locus ranges from 2 to more than 100. These loci are known as variable-number-tandem-repeats (VNTRs). Alec Jeffreys found two "core sequences" that were common to many of the repeated sequences. He discovered that probes for these core sequences hybridize to digest human DNA, creating distinctive banding patterns that are inherited in a Mendellian fashion. With the Jeffrey's multilocus probes or a well constructed cocktail of single-locus probes, the chances of two people having the same DNA fingerprint is about 1 in 3-30 billion [16]. Comparing DNA sequences to establish or rule out identity, relationship or ancestry is becoming common. The United Kingdom, where DNA profiling was pioneered, has for years collected DNA from all convicts. In USA, Virginia was the first state to establish such database. In 1992, the U.S. army began collecting blood and tissues from all new recruits as a part of a genetic" dog tag "program aimed at better identification of soldiers killed in combat".

An important limitation of DNA fingerprint analysis is that first it requires a relatively large sample of DNA (10,000 cells or about 50 ug), that is not usually found at a crime scene and secondly DNA should not be degraded. Forensic scientists have recently developed a different set of markers that are analyzed by PCR, allowing trace evidence samples to be typed. The markers, short tendem repeats (STRs) (microsattelites), are very similar to VNTRs. But the repeated motif is shorter-between two and nine base pairs. Thirteen tetrameric (four base pair repeat) STRs have been developed onto a marker panel (called CODIS panel) that is currently used by FBI to do DNA typing of crime suspects and create a database of the DNA profiles of convicted felons. It is also used routinely in forensic casework to generate DNA profiles from trace samples (e.g. single hair, saliva, blood and semen stains) and from samples that are old and or degraded. As a result, STRs have replaced VNTRs in most forensic labs. DNA profiling has also some positive effects and equally successful in overturning many conviction and brought freedom to many innocents.

Genetics as it is known today is largely the result of research performed during the 20th century. In 1903, Archibald Garrod [17] described alkaptonuria as the first "inborn errors of metabolism" and in 1909, Johannasen coined the term Gene to denote the basic units of heredity. The past several decades were the period of considerable experimental and theoretical work. Several organisms, including *Drosophila* (fruit fly) and *Neurospora* (bread mold) served as useful experimentation systems for studying the action and interactions of genes [18]. In 1931, Barbara McClintock and Harriet Creighton [19] provided a direct physical demonstration of recombination. By examining maize chromosomes microscopically, they could detect recombination between two easily identifiable features of

particular chromosomes. Shortly after this, Curt Stem observed the same phenomenon in Drosophila. H.J. Muller in 1926 discovered that x-rays induced genetic mutations in fruit flies 1500 times more quickly than under normal circumstance and same year Morgan published the chromosomal theory of gene. In 1944, Oswald Avery [20] showed that DNA was the right choice and the genes were composed of DNA. Erwin Charagaff in 1950 [21] found that in DNA, the amounts of adenine and thymine were about the same, as were the amounts of cytosine and guanine.

Although Friedrich Miescher discovered DNA in 1869 but the most significant achievements of 1950s was the discovery of its physical structure by James Watson and Francis Crick. These scientists provided the answer in 1953 by building models based on chemical and physical data that had been gathered in other laboratories, primarily x-ray diffraction data collected by Rosalind Franklin and Maurice Wilkins. Watson and Crick reported their discovery in a letter to journal *Nature* [22]. This paper that was published in the April 25, 1953 was a classic of simplicity-only 900 words, barely over a page long. Back to back were the paper by Wilkins and Franklin and their colleagues showing the x-ray data. Watson, Crick and Wilkins shared the Nobel Prize in 1962 for physiology and medicine. Rosalind Franklin who greatly contributed to the discovery of the double helical structure of DNA was unfortunate as she had died before this date and Nobel Prize rules do not allow a prize to be awarded posthumously. Another significant accomplishment of the decade was the correct number of human chromosome. Only in 1956 the correct number, 46 were finally determined [23]. The ability to count and identify chromosomes led to flurry of findings in cytogenetics, including the discovery of Down syndrome, caused by an extra copy of chromosome 21.

The advances in basic research in molecular genetics and technological development after 1960 have brought significant achievements at an ever increasing but at an unexpected rate. In 1958, Mathew Meselson and Franklin Stahl [24] demonstrated the semi-conservative replication of DNA; messenger RNA was discovered by in 1961 by Sydney Brenner and his colleagues [25]. Nirenberg and Khorana in 1960's deciphered the genetic code, demonstrating that a sequence of three nucleotide bases (codon) determined each of 20 amino acids [26, 27] for which they shared the Nobel Prize. In 1970, Hamilton Smith [5] discovered the restriction enzymes that can cleave DNA at specific sites, which made cutting and pasting easy thus facilitating DNA cloning [28]. All this became possible with availability of large number of molecular techniques that have been either perfected or were improved over a period of time. Of these, polymerase chain reaction (PCR) that was invented by Karry Mullis [29] has revolutionized both molecular diagnosis and molecular analysis of genetic diseases. PCR can selectively amplify a single molecule of DNA or RNA several million-fold in few hours. During the past two decades thousands of genes of interest have either been cloned or sequenced and have been mapped to specific chromosomes location. Clone, is a recombinant DNA molecule containing a gene or other DNA sequence of interest. Cloning is also the creation of a genetic replica of an individual. The technique transfers a nucleus from a somatic cell into an oocyte whose nucleus has been removed, then develop new cells from the manipulated cell. Ian Wilmut and others 1997 reported cloning of a sheep named Dolly, followed by a report of cloning a mouse named Cumulina, a cat named Cc a few farm animals and a six- celled human embryo [30]. This was the closest call to cloning of humans and certainly was a matter of ethical and religious concern. To the relief of many, Dolly died in February 2003 of lung disease. She was aging twice as fast than normal sheep, signaling the trouble for cloning.

The Human Genome Project (HGP) an international effort to map and sequence human DNA was launched in 1990. Two preliminary drafts of the physical map of human genome sequence were published in mid February 2001 issue of Science and Nature and in 2003 the complete human DNA sequence that coincided with 50^{th} anniversary of discovery of this eternal molecule was produced [31, 32]. Not all of genes have been identified but it appears that humans have about 30,000 genes distributed in 23 pairs of chromosomes. The average gene size, including introns and exons, is 27kb. Human genes tend to be largest and contain more and larger introns than the genes and introns of the invertebrate genomes such as Drosophila. The largest human gene (1.25Mb in length) is dystrophin gene that is associated with muscular dystrophy. HGP has contributed towards the knowledge of human genetics and as such important developments in computer technology have occurred that has helped to decipher the barrage of data that is being generated by HGP and related projects [33]. In addition to mapping genes, the molecular geneticists have pinpointed the molecular defects underlying a number of important genetic disorders whose number is now more than 6000. HGP has further contributed to our understanding of how gene defects can cause disease, opening paths to

more effective genetic testing, treatments and potential cures. In 2007 a world wide efforts called Genome–wide Association Studies has been started to understand the genomic variations among various individuals. This will help us to understand disease cause and some personal traits. [34]. Microarray technology is powerful tool in genetic research that utilizes nucleic acid hybridization techniques and recent advantages of genes within a single experiment [33]. Currently two main types of DNA microarrays are being used: oligonucleotide (usually 25-to 27- mers) arrays and gene expression arrays containing PCR products prepared from cDNAs. Software linked to the microarrays analyzes the pattern of hybridization and the data can be presented in several forms [35]. Bioinformatics is another emerging field that is providing the tools for analyzing the genomic information. We are now in the era of proteomics; a technology that involves separation and identification of protein isolated from cells shedding some information that how bacterial proteomates change with the alteration in the environment [36, 37].

It is now clear that genetics has an important role to play in future medicine. Increased understanding of the molecular basis of the human diseases has led to a number of potential therapies for various inherited disorders. Gene therapy is a technique in which genetic material is transferred to somatic cells of a patient to correct an inherited disease; or in other words, treatment of inherited disorders by insertion of normal gene. After successful trials in 1990's in different patients with several forms of severe combined immune deficiency (SCID), hemophilia A (factor VIII deficiency), Canavan disease and cystic fibrosis, it brought hope for many patients with inherited disorders. It was indicated that the somatic cell gene expression as shown by the long-term expression of the transferred genes would be practical and safer approach [38]. But there were many scientific and ethical concerns especially with which they can be prevented from replication [39]. Available data indicate that 632 gene therapy trials were underway worldwide before one mishap. Most trials involved cancer treatment (63%) and the retroviruses were the commonly used vector [34], followed by adenoviruses (27%) and lipofection (12%). Unfortunately, gene therapy trials took a worse turn when in September 1999, an 18 year old Jesse Gelsinger, a patient with SCID who received his first dose of gene therapy died because of multiple organs failure [40]. Some patients with SCID disease also reported to develop leukemia-like disease [41]. Following these incidents, U.S. Food and Drug administration halted all gene therapy trials to scrutinize them thoroughly. In 1998 Andrew Fire and Craig Mello discovered RNAi technology from *Caenorhabditis elengans* and now it has become an important research tool in biology to study the role of different genes in the pathogenesis of many diseases such as human cancers, cardio vascular disorders and others [42].

Attention has now been diverted to stem cell technology and many physicians are beginning to use stem cells to treat particular disease or injuries e.g. the scientists have taken adult stem cells from patients with chronic heart failure and injected them into their hearts, restoring normal function. But according to biologists, the most promising cells for therapy are embryonic stem cells. There are two sources of obtaining ES cells. One is the availability of frozen embryos from infertility clinics and second source is to create an embryo, using nucleus from a somatic cell from a patient (cloned embryo), such as a person who has suffered a spinal cord injury. Human embryonic stem cells, capable of morphing into any one of the more than 200 types in the human body, have become a wedge issue. Since currently the prominent source of obtaining ES cells is days-old embryo (rather from cloned embryos), this has raised serious ethical and religious issues. The fact that early embryos are destroyed in the process of establishing human ES cell lines has disturbed people who believe that pre-implantation embryos are person, with rights to live. But others believe that these embryos are too primitive to have an inherent moral issue [43]. The embryonic stem cell research although still is in infancy, but it has potentials to treat millions of people around the world who suffer from array of illnesses and conditions from Alzheimer's to spinal-cord injuries. The scientists believe that ES cells could be one of the greatest revolutions in modem medicine [44] but similar things of course were said for the gene therapy.

It is fair to say that cracking of DNA code has changed how we live, heal, eat and imagine the future, but one thing is clear that future decades promise to be a time of great excitement and fulfillment. On July 28, 2004, Francis Crick died at the age of 88. The contributions of late Rosalind Franklin (1920-58) are equally important because she was who provided a clue to Watson and Crick(45) that would prove pivotal in revealing the structure of DNA. In 2007, Mario Capecchi, Sir Martin Evans and Oliver Smithies got their Nobel Prize for their discoveries of principles of introducing specific gene modifications in mice by using embryonic stem cells. Their discoveries made it possible to carry out targeted

gene modifications in individual cells in a culture [46]. On October 26, 2007, Aurthur Konberg died at the age of 89 and his contribution in the field of enzyme biotechnology will be remembered indefinitely.

ACKNOWLEDGEMENTS

The information for the completion of this review were taken from different sources, including websites and work done by the authors listed in the references is fully acknowledged. I would also like to acknowledge the assistance provided by Mr. Rashid Hussain, Research Officer, Medical Genetics Lab, NCEMB in the preparation of this review article.

APPENDIX 1: BIOTECHNOLOGY TIME LINE

6000BC	Sumerians and Babylonians used yeast to make beer
4000BC	Egyptians bake leaven bread using yeast
	The preservation of milk by lactic acid bacteria resulted in yogurt.
	Molds were used to produce yeast.
	Fermentation was used to make vinegar and wine.
500BC	Chinese used moldy soybean cured as an antibiotic to treat boils.
100AD	Powered chrysanthemum was used in China as an insecticide.
1100-1700	Spontaneous generation is the explanation that organism arise from non-living matter.
1300	The Aztec harvested algae from lakes as a food source.
1590	Janssen invented the Microscope.
1665	Robert Hooke observed the cellular structure of cork.
1668	Francesco Redi disproved spontaneous generation and was one of the first to conduct a controlled experiment.
1660-75	Marcello Malpighi used the microscope to study blood circulation in capillaries.
	He described the nervous system as bundles of fibers connected to the brain.
1673	Anton Van Leeuwenhoek was the first to describe the protozoa and bacteria and recognized that they played a role in fermentation.
1701	Giacomo Pylarini practiced "inoculation" - intentionally giving children smallpox to prevent a serious case later in life.
1724	Cross-fertilization in corn was discovered.
1750	Farmers in Europe began rotating leguminous crops to increase yield.
1797	Edward Jenner inoculated a child with a viral vaccine to protect him from smallpox, intentionally infected humans with cowpox to induce resistance to smallpox.
1799	Lazaro Spallanzani tested the possibility of using heat to kill all microbes.
1809	Nicolas Appert devised a technique using heat to can and sterilize food.
1830	Proteins were discovered.
1833	The first enzymes were isolated.
1855	The Escherichia coli (E.coli) bacterium was discovered.
1856	Karl Ludwig discovered a technique for keeping animal organs alive outside the body, by pumping blood through them.
1859	Charles Darwin published "On the Origin of Species".
1863	Louis Pasteur invented the process of pasteurization, heating wine sufficiently to inactivate microbes, while preserving taste.
1864	Gregor Mendel advanced the principle of segregation and independent assortment.
	Joseph Lister began using disinfectant in wound care and surgery.
	Louis Pasteur developed the germ theory.
1869	Friedrich Miescher discovered DNA in the sperm of trout.
1870	W. Flemming discovered mitosis.
1871	Ernst Hoppe-Seyler discovered invertase, an enzyme that cuts the disaccharide sucrose into glucose and fructose.
	A technique for growing, staining and identifying bacteria was developed by Robert Koch.
1878	Laval designed the first centrifuge.
	Joseph Lister described the first method for the isolation of pure cultures of bacteria.
1879	Flemming discovered chromatin, the rod-like structure inside the cell nucleus that later was called chromosomes.
	William Beal made the first clinically controlled crosses of corn in search of colossal yields.
	Albrecht Kossel began his studies of nucelin, leading to his discovery of nucleic acids.
1881	Robert Koch made nutrient agar a standard tool for obtaining pure culture and for identifying genetic mutants.

Pasteur used attenuation to develop vaccines against bacterial pathogens of fowl cholera and anthrax.

1882 Flemming reported his discovery of chromosomes and mitosis.

Koch became the first to uncover the cause of a human microbial disease, tuberculosis and published that specific diseases were caused by specific organisms.

1884 Koch stated his "postulates" for testing whether a microbe was a causal agent of a disease.

Pasteur Gram described the differential staining technique for bacteria known as the Gram stain.

1885 Pasteur began human trials of his rabies vaccine.

1885-95 Koch, Petri, Loffler, Yersin and Erlich identified a host of human disease causing organisms. Emil von Behring developed the first antitoxin for deptheria.

1886 Emil von Beneden discovered that each species has a fixed number of chromosomes; he also discovered the formation of haploid cells during cell division of sperm and ova (meiosis).

R. J. Petri described circular plates with overlapping glass lids (Petri-dish) for growing microbes on nutrient agar.

1892 Ivanonsky described viruses as the causal agent of the tobacco mosaic disease.

1896 Wilhelm Kolle developed cholera and typhoid vaccines.

1897 Friedrich loeffler and P. Frosch reported the pathogen of foot and mouth disease of cattle to be a virus.

Ronald Ross discovered Plasmodium, the protozoan that causes, in the Anopheles mosquito and showed the mosquito transmits the disease agent from one person to another.

1900 Drosophila (fruit fly) was used in early studies of genes.

Walter Reed established that mosquitoes transmitted yellow fever, the first viral human disease.

Hugo De Vries, Elrich von Tschermak and Carl Correns all independently confirmed Mendel's work.

William Sutton observed homologous pairs of chromosomes in grasshopper cells.

Major outbreaks of disease in overcrowded industrial cities led to the introduction of large scale sewage purification system based on microbial activity.

1902 Archibald Garrod first suggested a genetic cause of for a human disease.

Walter Stanborough Sutton stated that chromosome were paired and may be the carriers of heredity.

The term "immunology" first appeared.

1903 William Sutton and Theodore Boveri proposed the chromosome theory.

1904 William Bateson demonstrated that some characteristics are not independently inherited, introducing "gene linkage".

1905 Edmud Wilson and Nellie Stevens showed that a single Y-chromosome determined maleness and two copies of X-chromosome determined femaleness.

1905-08 The term "Genetics" was introduced. It was demonstrated that some genes modify the actions of other genes.

1906 Paul Erlich investigated atoxyl compounds and discovered the beneficial properties of Salvarsan-the first chemotherapeutic agent.

1907 Calmette and Guerin developed a vaccine against TB, called BCG it was not used until 1921.

A. E. Gattod discovered role of genetics in biochemistry. He described *"Inborn errors of Metabolism"* based on his analysis of family medical histories.

1908 Wilhelm Johannsen coined the term "Gene" to describe the carrier of heredity; genotype to describe the genetic constitution of an organism and phenotype to describe the actual organism, which results from a combination of genotype and the various environmental factors.

Pheobus Levene discovered that sugar ribose was present in some nucleic acids, RNA.

1910 Thomas Morgan proved that genes are carried on chromosomes.

1911 Rous discovered the first cancer-causing virus.

Morgan began to map the position of genes on chromosomes of the fruit fly. Structure of simple crystalline substances.

1913 Alfred Sturtevant constructed first gene map of Drosophila.

1914 Bacteria were used to treat sewage for the first time in Manchester (UK).

1915 Frederick Twort discovered the phages, or bacterial viruses.

1916 George Shull, a pioneering corn breeder and Princeton geneticist published the inaugural issue of Journal of Genetics.

1917 plough demonstrated the rearrangement of chromosomes known as "crossing over".

1919 A Hungarian agriculture engineer used the word "Biotechnology".

1920 Evans and Long discovered the human growth hormones.

1921 Hermann Muller described genes as particles that despite their ultramicroscopic size exhibit a complex structure of different parts.

1926 Morgan published "The Theory of the Gene".

Muller discovered that x-ray induced genetic mutation in fruit flies 1500 timesn faster than under normal circumstances.

1928 Alexander Fleming discovered the penicillin, the first antibiotic.

Louis Stadler showed that UV radiation could also cause mutations.

Fredrick Griffiths noticed that "transforming principle" could change a rough type of bacterium to a smooth type- this was later identified as DNA.

1928-35 Linus Pauling elucidated the physical laws governing how atoms were arranged in molecules and also described sickle cell anemia, a molecular disease.

1929 Levene discovered an unknown sugar, deoxyribose

1931 Barbara McClintok and Harriet Creighton provided a direct physical demonstration of recombination by examining the maize chromosomes microscopically.

1935 Wendell Stanley crystallized the tobacco mosaic virus, the first purification of a virus.

Andderi Belozerssky isolated DNA in the pure state for the first time.

1936 Stanely isolated nucleic acids from the tobacco virus contained RNA.

1938 The term "Molecular Biology" was coined.

1939 Belozersky showed that both DNA and RNA were always present in bacteria.

1940 Oswald Avery demonstrated that DNA is the "transforming factor" and was the material of genes.

1940-45 Large-scale production of penicillin was achieved.

1941 Jost, a Danish microbiologist first used the term "Genetic Engineering". George Beadle and Edward Tatum developed the "one-gene-one-enzyme" hypothesis.

1942 The electron microscope was used to identify and characterize a bacteriophage-a virus that infects bacteria.

1943-53 Cortisone was first manufactured in large amount.

1944 Waksman isolated streptomycin, an effective antibiotic for TB.

Oswald Avery, Collin Macleod and Maclyn McCarthy determined that DNA was the hereditary material involved in transformation in pneumoccus bacteria.

Fredrick Singer developed chromatography to determine the amino acid sequences of bovine insulin molecule.

1944 Lauria and Delbruck developed a simple model system using phage, to studythe transfer of genetic information to host bacterial cells.

1945-50 Isolated animal cell cultures were grown in laboratories.

1945 Max Delbruck and Alfred Hershey discovered that genetic material from different virus could be contained to form a new type of virus, an example of genetic transformation.

1950 Artificial insemination of livestock using frozen semen was successgully accomplished.

Erwin Charagaff found that in DNA the amount of adenine and thymine were about the same as the amounts of cytosine and guanine.

1951 Esther Lederberg discovered lambda phage, a virus of E.coli. Linus Pauling deciphered the structure of the protein keratin.

1952 jousha Lederberg and Norton Zinder showed that bacteria sometimes exchanged genes by an indirect method which they called "transduction".

1953 Linus Pauling concluded that DNA was three stranded molecule with the sugar phosphate backbone at the center.

Nature published Watson's and Francis Crick's manuscript describing the double stranded helical, complementary, anti-parallel structure of DNA.

George Gamow suggested that DNA hold the code for making proteins.

1954 Cell culturing techniques were developed.

1955 An enzyme involved in the synthesis of a nucleic acid was isolated for the first time.

Seymour Benzer devised an experimental set up to map mutations with a short genetic region of a particular bacterial virus.

1956 The fermentation process was perfected in Japan.

Coenberg discovered the enzyme DNA polymerase 1, leading to an understanding of DNA replication.

1956 Francis Crick and George Gamov worked out the "central dogma", explaining how DNA functions to make proteins.

Mathew Meselson and Francis Stahl demonstrated the replication mechanism of DNA.

1958 Sickle cell anemia was due to a single amino acid in B-globin chain.

1959 The first human chromosome abnormality Down syndrome was identified. Francis Jacob and Jacques Monod established the existence of genetic regulation, mappable control functions located on the chromosomes in the DNA sequences, which they named the repressor and the operon.

1960 Exploiting base pairing, hybrid DNA-RNA molecules were created.

Sydney Brenner, Francois Jacob, Matthew Meselson discovered messenger RNA.

1961 Marshall Nirenberg discovered that UUU was the codon for phenylalanine, the first of 64 letters genetic code for proteins.

1962 Crick, Watson and Wilkins won Nobel Prize for physiology and medicine.

1964 The International Rice Research Institute in Philippines introduced the new strains of rice, starting the Green Revolution.

1965 Harris and Watkins successfully fused mouse and human cells.

Scientists noticed that genes conveying antibiotic resistance in bacteria were often carried on small supplementary chromosomes called plasmids.

1965 Nirenberg and Khorana cracked the genetic code, demonstrating that a sequence of three nucleotide base (codon) determines each of the 20 amino acids.

1967 A first automatic protein sequencer was perfected.

Mary Weiss and Howard Green published a technique called somatic cell hybridization.

1969 A Harvard medical school team isolated the first gene, a segment of bacterial DNA that played a role in sugar metabolism.

1970 Hamilton Smith discovered restriction enzymes that cut DNA at specific sites that facilitated DNA cloning.

Peter Vogt and Peter Duesberg discovered the first oncogene in a virus, SRC.

1972 Paul Berg made the first DNA in vitro.

The first successful DNA cloning experiments were performed in California. The DNA composition of humans was discovered to 99% similar to that of chimpanzees and gorillas. Initial work with embryo transfer took place.

1973 Stanely Cohen and Herbert Boyer first used a plasmid to clone DNA.

Bruce Ames discovered that cancer-causing chemicals also can cause mutations in DNA, the basis of the Ames test for carcinogenesis.

The first gene mapping conference took place.

1974 The NIH formed a Recombinant DNA Advisory Committee to oversee recombinant genetic research.

Cohen and Boyer published their work, expression of foreign gene implanted bacteria by recombinant DNA methods.

1975 The scientists met at the Asilmor conference center in California and called for guidelines regulating recombinant DNA research.

The first monoclonal antibodies were produced.

1976 J. Michael Bishop and Harold Varmus showed that oncogenes appeared on animal chromosomes and alteration in their structure or expression could result in cancerous growth.

The NIH released the first guidelines for recombinant DNA research.

The tools of recombinant DNA were first applied to a human inherited disorder.

Molecular hybridization was used for the parental diagnosis of alpha thatlassemia.

Scientists showed that the Yeast genes were expressed in E.coli bacteria.

1977 First expression of human genes in bacteria, somatostatin was demonstrated.
Bill Rutter and Howard Goodman isolated the genes for rat insulin.
Walter Gilbert and Frederick Singe* separately developed the methods for sequencing DNA.
Phillip Sharp, Richard Roberts and others identified interruptions (introns) in genes.

1978 Frederick Singer determined the sequence of an entire viral gene (0X174).
Recombinant human insulin first produced.
The viral coat protein in hepatitis B was cloned.
Genetech scientists cloned the gene for human insulin.

1979 Human growth harmone was first synthesized.

1980 Court allowed Exxon oil company to patent an oil-eating microorganism. The U.S. patent for gene cloning was awarded to Cohen and Boyer.
The first gene synthesizing-machines were developed.
Researchers successfully introduced a human gene-one that coded for the protein interferon into a bacterium.

1981 Martin Cline and CO-workers created a transgenic mouse by transferring functional genes from one animal to another.
Chinese scientist became the first to clone a fish- a golden carp.
A yeast expression system was made to produce the hepatitis B surface antigen.
Mary Harper and her colleagues mapped the gene for insulin.
Mapping in situ hybridization became a standard technique.

1982 Stanely Prusiner discovered prions, the infectious proteins responsible for scrapie and mad-cow disease.
Thmosa Cech and Later Sidney Altaian showed that RNA can act as an enzyme.
Genetically engineered human insulin was marketed.
Michael Smith developed a procedure for making precise amino acid changes anywhere in a protein.
First commercial gas phase protein sequencer was introduced.

1983 Kary Mullis developed the Polymerase Chain Reaction (PCR).
Monoclonal antibody-based diagnostic test for Cglamydia trachyomatis was introduced.
The artificial chromosome was synthesized.
The first genetic markers for specific inherited diseases were found.
U.S. patents were granted to companies for genetically engineered plants.

1984 Alee Jeffrey developed fingerprinting- using DNA for positive identification of individuals.
Elizbeth Blacburn and Greider discovered, telomerase, an enzyme that extendedthe life of cells.
The first genetically engineered vaccine was developed.

1985 Robert Gallo and Luc Montagnier independently published the genetic sequences of 11IV, an AIDS virus.
Genetic markers were found for kidney disease and cystic fibrosis.
Genetic fingerprinting was presented as evidence in the courtroom in UK.
Genetically engineered plants resist to insects, viruses and bacteria were field tested for the first time in USA.
NIH approved guidelines for performing experiments in gene therapy on humans.

1986 Leroy Hood invented the first genetically engineered vaccine for hepatitis B.
University of California, Berkley chemist described a method to combine
antibodies and enzymes (abzymes) to create pharmaceuticals.
First field tests of genetically engineered plants were conducted in USA.

1987 Allan Wilson, Rebecca Cann and Mark Stoneking determined that all living humans shared a common ancestor "Mitochondrial Eve".
First field trials of genetically altered bacterium "Frostban" that inhibits frost formation on crop plants were conducted on strawberry and potato plants in California.

1988 Harvested molecular geneticists were awarded the first U.S. Patent for a genetically altered animal transgenic mouse.
A patent for the process to make bleach resistant protease enzymes for use in detergents was awarded.

1989 The first genetic screening test (to determine sex) was performed on embryos before they were implanted in the uterus.

Field trials of a recombinant viral crop protectant were identified in USA.

1990 The Human Genome Project, an international collaborative program to map the entire genome and, ultimately, to determine its base sequence was launched.

"Chy-Max" an artificially produced from chymosin, an enzyme for cheese making was introduced, first application recombinant technology in food industry.

The first federally approved gene therapy trial using a retrovirus vector carrying ADA gene was performed successfully on a four year old girl suffering from an immune disorder was performed.

The first successful field trial of genetically engineered cotton plants was conducted. The plants had been engineered to withstand use of the herbicide "Bromoxynil".

The first *transgenic* dairy cow-used to produce milk proteins for infant formula was created.

1991 American and British scientists established a technique for testing embryos *in vitro* for genetic abnormalities such as cystic fibrosis and hemophilia. U.S. Army introduced a "genetic dog tag" program aimed at better identification of soldiers killed in combat.

1993 The Huntington's disease gene was identified.

Kary Mullis won the Nobel Prize in Chemistry for inventing PCR.

The U.S. FDA declared that the genetically engineered foods were "not inherently dangerous" and did not require special regulation.

1994 The FDA allowed the first genetically modified food product to market, *thoflavsavr* tomato, but a bland taste and high price made it a commercial dud.

The first breast cancer gene was discovered.

Genetically engineered version of human DNAase, which breaks down protein accumulation in the lungs of CF patient, was approved.

1995 DNA fingerprinting played an important roe-O. J. Simpson murder trial.

Craig Venter and Hamilton Smith the base sequence of the genomes of two free living organism, the bacterium *Hemophilus influenzae* and *Mycoplasma genitalium.*

DNA microarrays were invented.

Mutations in the BRCl and BRC2 genes were linked to hereditary breast ovarian prostate cancers.

Gene therapy, immune system modulation and genetically engineered antibodies were used fort cancer treatment.

First baboon-to-human bone marrow transplant was performed on an AIDS patient.

1995 Gene associated with Parkinson's disease was discovered, opening a new era for research into neurological disorders.

Many investigators determined the base sequences of brewer's yeast, *Sacchromyces cerevisiae,* the first eukaryotic genome to be sequenced.

1997 Ian Wilmut and others reported cloning a sheep, named Dolly, from an adult sheep udder cells.

A new technique combines PCR, DNA chips and a computer program providing a new tool in the search for disease-causing genes became available.

1998 Two teams grow embryonic stem cells in Petri dish.

University of Hawaii scientists cloned three generations of mice from nuclei of adult ovarian cumulus cells.

Embryonic stem cells to regenerate tissues and create disorder mimicking diseases. Emerged a viable tool for treatment of genetic disorders.

Scientists at Japan's Kinki University cloned eight identical claves using cells taken from a single adult cow.

The first complete animal genome for the C. *elegans* roundworm was sequenced.

A rough draft of the human genome map was produced, showing the locations of more than 30,000 genes.

1999 First known American death caused by gene therapy.

Many investigators determined the base sequence of human chromosome 22.

Potrykus and Beyer created a strain enriched with beta-carotene.

2000 The base sequence of the genome of fruit fly, *Drosophila melongaster* (a mainstay of genetic research) was determined.
Alan Fischer and colleagues performed first clearly successful gene therapy trial on two patients with SCID disorder.

2001 First work draft of human genome sequences was produced.

2002 Scientists at Texas A & M University cloned a house cat, named cc.
Gene therapy trials in Europe and USA for SCID were stopped after a child received the treatment developed a leukemia-like disease.

2003 Dolly, the cloned sheep died.
50th anniversary of Watson and Crick discovery of the double helix.

2004 Francis Crick died at the age of 88.

2005 Genomics, Bioinformatics, Proteomics, DNA Microarray Technology-diagnostic tool of genetic testing.

2006 Metagenomics and pyrosequencing played their role in evolutionary biology.

2007 Studies of genome variation and their role in disease and personal traits.
Preparation of induced Pluripotent Stem (IPS) cells.

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