



Biotechnology as a Cradle of Scientific Development: A Review on Historical Perspective

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SMA, AYK and SA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AMM and ASA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Historically, the science of biotechnology started through domestication of plants and animals by early men and selective plant breeding which subsequently led to transgenesis. Fermentation of grains and fruits using yeast was initiated in Egypt and other ancient parts of the world about 2500 BC. The practice of quarantining people to prevent the spread of diseases was introduced long before the origin of diseases. Introduction of traditional medicine was carried out by the ancient Egyptians using honey to treat infections until 1928 when Alexander Fleming extracted penicillin. Furthermore, synthetic antibiotic chemotherapy began in Germany with Paul Ehrlich in 1880s. The discovery of genes as a unit of inheritance was celebrated in 1865 by Gregor Mendel. Moreover, it took another 90 years of research before scientists discovered that genes were made up of DNA

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which was the beginning of modern biotechnology. The recombinant DNA technology was emerged in 1970s. These discoveries together with the new findings in all fields of biosciences and computer sciences gave rise to the modern day genetic engineering; as a result, cloning in 1980s, polymerase chain reaction in 1983 and DNA fingerprinting technique were developed respectively. Wide ranges of therapeutic proteins and biopharmaceutical products such as insulin, interferon vaccines, synthetic hormones, and synthetic antibodies were archived. Bioconversion of organic wastes by the use of genetically altered bacteria has gained considerable attention. Hence, biotechnology has greatly promoted human life socially, economically, scientifically and medically from primitive to modern and advance level.

Keywords: Biopharmaceuticals; cloning; DNA; fermentation; genetic engineering; transgenesis.

1. INTRODUCTION

Biotechnology is defined as any technique that uses living organisms or their parts to make or modify products, improve plants or animals, or develop microorganisms for specific uses to benefit people [1]. The concept encompasses a wide range of procedures for modifying living organisms that employ artificial selection and hybridization [2]. Since 2500 BC, biotechnology has been in practice for use to bake bread, brew alcoholic beverages and breed food crops or domestic animals. Recent development in molecular biology has given biotechnology new meaning, prominence and potential [3]. Biotechnology provides foundation for the engineering of bioprocesses which consequently promote the production of various human and animal healthcare products, food products, biologically active proteins, chemicals and biofuels. Industrial bioprocessing comprises the design and scale up of bioreactors that generate large quantities of transformed microbes or cells and their products as well as technologies for recovery, separation and purification of those products. It also helped in the development of

pharmaceuticals, foods, agricultural processes and gene therapy [1]. The historical development of biotechnology can be reviewed chronologically via the following biotechnological events and practices (Table 1).

2. DOMESTICATION OF ANIMALS AND PLANTS

Domestication began over 10,000 years ago when our ancestors started keeping plants as reliable sources of foods. Rice, barley and wheat were among the first domesticated plants [4]. Wild animals were tamed to provide milk or meat or help with ploughing or guarding. The dogs, sheep and goats were thought to be among the first domesticated animals [4]. The origin of domestication involves changes in organisms and human behavior. Differences between domestic plants and their wild ancestors evolved as a result of wild plants being selected, gathered and brought back to camp by hunter-gatherers while the roots of animal domestication include the ubiquitous tendency of all people in trying to tame or manage wild animals including ospreys, hyenas and grizzly bears which are the

Table 1. Chronological events in biotechnology

| Year | Scientist | Event |
|-------------|-------------------|---------------------------------------|
| 1963 | Robert Hook | Description of cells |
| 1675 | Van Leewenhoek | Discovery of protozoa and Bacteria |
| 1855 | Louis Pasteur | Discovery of yeast as living organism |
| 1888 | Waldye | Discovery of Chromosomes |
| 1928 | Alexander Fleming | Discovery of Penicillins |
| 1953 | Watson and Crick | DNA Three dimensional structure |
| 1973 | Cohen and Boyer | Recombinant DNA experiment |
| 1975 | Köhla and Cesar | Discovery of monoclonal antibody |
| 1983 | Kary Mullis | PCR technique discovery |
| 1990 | French Anderson | First Trial of gene therapy |
| 1997 | Ian Wilmut | Cloning of Dolly Sheep |
| 2010 | Craing Venter | Creation of first synthesized cell |

Source: [5]; <https://www.bio.org/articles/history>

unlikely candidates. Although humans have been manipulating wild plants and animals for long time, hunter-gatherer behavior began to change at the end of the Pleistocene because of increasingly unpredictable climates, decreases in big-game species that were hunters' first-choice prey and increasing human occupation of available habitat [6]. The Middle East was the first place where people domesticated plants (ca. 8300 BCE) and animals (ca. 7500 BCE) [7]. However, domestication of plants and animals took place independently in different parts of the world [7].

3. SELECTIVE PLANT BREEDING AND TRANSGENESIS

No doubt man selects varying individuals, sows their seeds and again selects their varying offspring; man therefore may be said to have been trying an experiment on a gigantic scale which nature during the long lapse of time has incessantly tried [8]. Eventually, people transported some wild plants from their natural habitats to more productive one and began intentional cultivation [6]. Crop improvement by selecting seeds from the most successful or healthiest plants to obtain a new crop with more desirable traits is a form of early crop technology. Farmers realized that using only the seeds from the best plants would eventually enhance and strengthen the desired traits. Gregor Mendel's studies on inheritable traits of peas in the mid 1860 have improved our understanding of genetic inheritance that led to practices of cross-

breeding popularly known as hybridization [9]. Diligent selection over the years resulted in crop genotypes which are suitable for human sustenance [10]. Conventional plant breeding has its own limitations due to non-availability of sources of resistance to pests and diseases in crop germplasm. In addition, introgression of resistance from wild and weedy relatives has hampered by problems of incompatibility. It is now possible to introduce the genes encoded with valuable agronomic traits from any biological organism into the crop plants. Techniques are available to incorporate foreign genes (transgenes) into other organisms' systems with the precision and reliability [10]. The first transgenic animal (Chimeric mice) was produced in 1970s before the term transgenic was first used by J.W. Gordon and F.H. Ruddle in 1981 [11]. Transgenic plants were first created in the early 1980s by four groups working independently at Washington University that produced cells of *Nicotiana plumbaginifolia*; a close relative of ordinary tobacco that was resistant to the antibiotic kanamycin [12]. The transgenic plants carrying traits for insect resistance, nutritional quality, viral resistance and disease resistance are still under development [10]. Transgenic animals are more widely used for various purposes which include obtaining information on gene function regulation and human diseases, high value recombinant pharmaceutical proteins and xeno-organs for human products to be used for human therapy and also improving animal products for human consumption [13].



Fig. 1. Genetically modified foods
source: [47]

4. FERMENTATION

Fermentation is the chemical transformation of sugars into simpler compounds like acids, gases and alcohols by the action of enzymes which are complex organic catalysts originated from molds, yeast or bacteria [14]. Fermentation of grains and fruits to alcoholic beverages was carried out in Egypt and other parts of the ancient world in about 2500BC. Other types of food fermentation practiced for thousands of years include the transformation of milk into cheese and fermentation of soy beans. However, it was not until 1857 that Pasteur proved that alcoholic fermentation was caused by living cells [1]. Buchner won the Nobel Prize in 1907 for producing a cell-free extract of yeast cells which could ferment sugar showing that presence of living yeast cells is not necessary for fermentation whereas enzymes are responsible [15]. In the ensuing 100 years, the intentional manipulation of microbial fermentations to obtain food products, solvents, beverages and later substances that are having therapeutic value such as antibiotics gave rise to a large scale fermentation Industry. Furthermore, modern biotechnology began to be applied in developing advanced enzymes for converting cellulosic materials to fermentable sugars. This process of engineering to improve grain-to-ethanol plants and the rapid build out of an expanded ethanol industry began which provided the renewable liquid fuels in small but significant quantities [1]. In 1878 K'uhne introduced the term 'enzyme' from the Greek *enzumos*, which refers to the leavening of bread by yeast. A clear understanding of wide range of enzymes present in living cells and their modes of action happened with the development of the science of biochemistry. Although enzymes are only formed in living cells but many can be extracted or separated from the cells and can continue to function *In vitro*. This unique ability of enzymes to perform their specific chemical transformations in isolation has led to an ever-increasing use of enzymes in industrial, food processing, bioremediation and medicine; their production is collectively termed as enzyme technology [16]. Cellulase enzymes were introduced in the 1980s as a denim-washing aid to achieve a faded and abraded look similar to that provided by pumice stones. Cellulase works by loosening the indigo dye on the denim in a process known as 'biostonewashing'. A small dose of enzyme can replace several kilograms of pumice stones [3]. [17] used fermentation as an appropriate technique to convert slaughterhouse

blood into nutritionally sound and microbiologically safe ingredient for the use in fish feed formulation.

5. ANCIENT PRACTICES OF QUARANTINING PEOPLE

The word quarantine was derived from the Italian words *quaranta* and *giorni*, meaning 40 days [18]. It refers to all the measures taken to minimize the risk of infectious diseases caused by viruses, bacteria or other micro-organisms entering and establishing potential harm to the population, food security and economy [19]. The practice of quarantining to prevent the spread of diseases had started long before the origin of the diseases were known. However, it demonstrates early acceptance that illness could be passed from an infected individual to healthy individual who would then begin to have symptoms of the disease [9]. The practice of quarantine began during 14th century in an effort to protect coastal cities from plague epidemics. Ships arriving in Venice from infected ports were required to sit at an anchor for 40 days before landing. Now a days, a case, carrier, suspected case or suspected carrier of diphtheria, rubella, influenza with pandemic potential, invasive meningococcal disease, measles, monkey pox, mumps, pertussis, poliomyelitis, pneumonic form of plague, severe or novel coronavirus, vancomycin intermediate or resistant *Staphylococcus aureus* (VISA/VRSA), smallpox, tuberculosis (active), vaccinia disease, viral hemorrhagic fever or any other contagious disease that may pose an imminent and significant threat to the public health can be quarantined [20].

6. TRADITIONAL MEDICINE AND CHEMOTHERAPY

The ancient Egyptians used honey for respiratory infections and as an application for wounds; it is natural antibiotic used to prevent wounds from becoming infected. By about 600 BC, the Chinese were using mouldy soy bean curds to treat boils. Similarly, Ukrainian peasants were using mouldy cheese to treat infected wounds. The moulds released natural antibiotics that killed bacteria and prevented the spread of infection. Despite these natural treatments, it wasn't until 1928 that Alexander Fleming first extracted penicillin, the first antibiotic from mould [4]. Synthetic antibiotic chemotherapy as science and development of antibacterial agents began in Germany with Paul Ehrlich in late 1880s [21].

7. FOOD PROCESSING TECHNOLOGY

Food processing is the transformation of raw ingredients by physical or chemical means into food or conversion of food into ready to use forms; it also combines raw food ingredients to produce marketable food products that can be easily prepared and served by consumer [22]. To decrease the risk of unpredictable variation in food supply, people broadened their diets (the so-called broad-spectrum revolution) to second and third choice foods which included more small game plus plant foods requiring much preparation such as grinding, leaching and soaking [6]. Nicolas Appert was awarded for inventing canning in 1810. Furthermore, Ice berg lettuce which is state of the art refrigeration technology was invented in 1910, in 1912 also, Louis camille Maillerd discovered many flavors, odors and colors that makes food inviting as a result of heating amino acids and sugars (Maillerd reaction). A research center for the study of bacteriology of food spoilage was first launch in 1913 [23].

8. THE DISCOVERY OF GENES

In past centuries, it was customary to explain inheritance by saying, "it's in the blood." People believed that children received blood from their parents and that a union of bloods led to the blending they saw in one's characteristics. Such expressions as "blood relations "blood will tell," "bloodlines" reflect this belief [24]. A monk named Gregor Mendel identified genes as the unit of inheritance in 1865. It took another 90 years of research before scientists discovered that genes were made up of DNA. This discovery was the beginning of modern biotechnology [4]. Mendel postulated that an inherited trait is controlled not by blood but by "factors" obtained from the parents. His work with pea plants implied that one factor is obtained from each parent and that a particular factor may dominate the other. It also appeared that the factors separate during transmission to the next generation. However, at that time, little attention was paid to the work. At the beginning of the 1900s, Mendel's experiments were repeated and verified; later scientists postulated that his "factors" were really chromosomes. Morgan's work of 1910 showed that white eye color in fruit flies is determined by a single chromosome and he postulated that a single chromosome determines a trait. But individual chromosomes could not explain all traits; molecular geneticists came to believe that entities on the chromosome

called "genes" were the hereditary factors. Evidence presented by Miescher, Feulgen, and Mirsky indicated that chromosomes are composed of deoxyribonucleic acid (DNA). Experiments performed by other biologists and chemists strengthened the link between genes and DNA. Griffith showed that the characteristics of certain bacteria could be changed (i.e., the bacteria could be transformed) if they were mixed with debris from another type of bacterium in 1928. Alloway and his group found that purified debris could increase the potentiality of bacterial transformation. Avery and his colleagues discovered that the transforming material in the debris was DNA. The experiments of Hershey and Chase provided the final proof for the involvement of DNA in heredity. They performed experiments with bacteria and the viruses that replicate within it. Bacterial viruses are composed primarily of protein and DNA. The experimental results showed that DNA alone directs the replication of new viruses which implies that DNA contains the biochemical information for the synthesis of both the viral protein and the viral DNA. The results obtained by Hershey and Chase confirmed that DNA is the hereditary material and stimulated additional interest in the study of molecular genetics [24]. By 1900, it was known that the basic building blocks of DNA were phosphate, a sugar (later shown to be deoxyribose) and four heterocyclic bases - two of which were purines [adenine (A) and guanine (G)] while the other two were pyrimidines [cytosine (C) and thymine (T)]. Levene also postulated that the amount of the four bases were the same in all DNA molecules whatever their origin. In the 1930s, Torbjörn Caspersson and Einar Hammersten showed that DNA was a polymer but still most people continued to believe in Levene's 'tetra nucleotide hypothesis'. A visit to the Cavendish by Chargaff in 1952 prompted the further thought that perhaps the sequence of bases might represent the genes in a chemical code. Meanwhile, Pauling published a paper on the DNA structure but it contained a major error (he put the phosphate groups on the inside). The entry of this scientific giant into the race spurred Crick and Watson to greater efforts. The discovery of DNA structure by James Watson and Francis Crick in 1953 has transformed the life sciences from biochemistry and agriculture, to medicine and genetics. However, it also leads to the new era in biology bringing about the cracking of the triplet genetic code by Har Gobind Khorana and the realization that DNA directs the synthesis of proteins. More recently,

the complete sequences of many organisms have been solved - including the human genome in June 2000 [25]. It was a dream of researchers to replace the defective genes with good ones and cure genetic disorders. Undoubtedly, the most far-reaching and controversial area of genetic engineering of humans is gene therapy [26]. Gene therapy is the process of inserting genes into the cells to treat diseases. The newly introduced gene will encode protein and correct the deficiency that occurs in genetic diseases. Thus gene therapy is primarily involves the genetic manipulation in animals or humans to correct a disease and keep the organisms in good health [27]. The first trial of gene therapy was started in 1990 by French Anderson [5].

9. RECOMBINANT DNA TECHNOLOGY/ GENETIC ENGINEERING

The modern biotechnology began to emerge in the late 1970s when recombinant microorganisms began to use for making high value proteins and peptides for biopharmaceutical applications. This effort evolved into the production of some key life saving proteins and the development of monoclonal antibodies that subsequently proved to be effective molecules in the fight against

cancer [1]. Precisely, the first experiment in which the DNA fragments were joined *In vitro* and recombinant molecules were introduced into living cells were performed in 1973 by Boyer and Cohen. Two years later, the basic technology for the production of monoclonal antibodies from a single cell was established. This information together with new findings in all fields of biosciences and computer science gave rise to the modern day genetic engineering [29]. Although in principles, a gene of any size can be made chemically but is often easier to isolate it naturally after full sequencing to “edit” the message by constructing nucleotides with specific changes. However, another major impact of *In vitro* recombinant DNA technology leads to understanding of the basic causes of diseases [30].

Cloning is a process of cutting and pasting of DNA from its original genome into a convenient carrier DNA called vector to produce an identical copy called clone; it involves the use of restriction enzymes and transfer of DNA from one organism to another [24]. Cloning of mammals via nuclear transfer was initially reported in mice in early 1980s, approximately 30 years after the first tadpole clones were produced [31]. In 1997, scientists in Scotland have announced the birth of the world's first

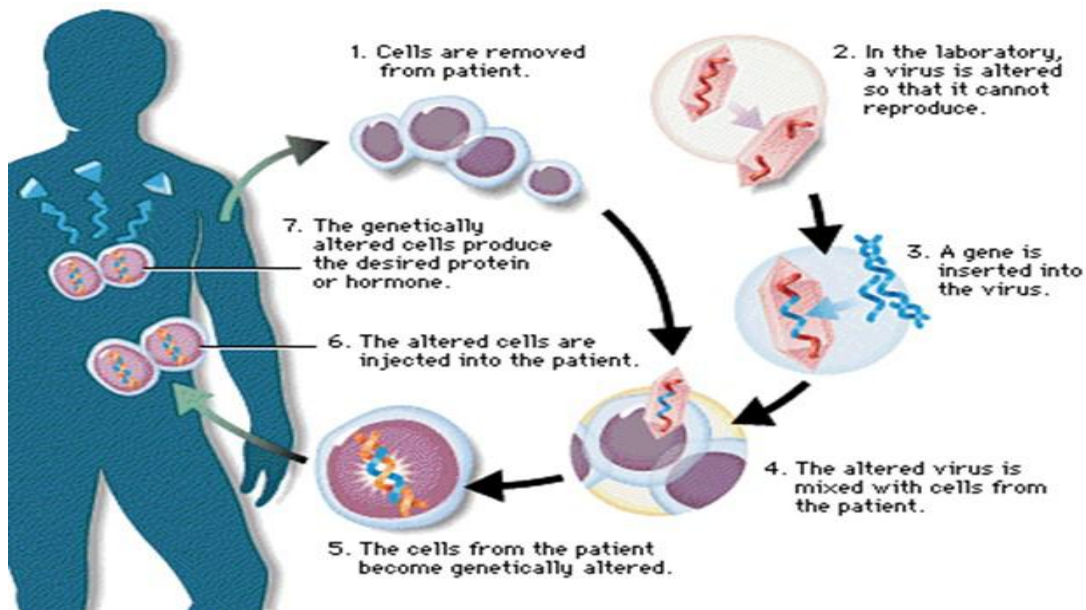


Fig. 2. Steps involved in gene therapy.

Source: [28]

successfully cloned mammal from adult cell "Dolly the sheep". Dolly was created at the Roslin Institute in Edinburgh, was born on 5th July 1996 [32]. Polymerase chain reaction (PCR) was developed by Kary Mullis in 1983 which allowed the manipulation of DNA. The DNA fingerprinting technique was developed in 1984 which allowed a piece of DNA to be replicated over and over again *In vitro* that led to its understanding more [16]. A wide ranges of therapeutic proteins and the use of genetically engineered pharmaceutical products such as insulin, interferon, hepatitis B vaccine and other growth hormones were achieved during 1980 to 1985 [30]. In 1989, a research group at the University of Washington filmed DNA molecules moving through an agarose gel have resulted to DNA agarose gel electrophoresis. The length and purity of DNA molecules can be accurately determined by the agarose gel electrophoresis [33,34].

In the late 1970s, methods were developed that allowed the nucleotide sequence of any purified DNA fragment to be determined simply and quickly. This made it possible to determine the precise order of nucleotides within DNA molecule of tens of thousands of genes. The process is now commonly known as DNA sequencing [33]. In the late 1980s and early 1990s biotechnology found further application in sequencing of the human genome together with the sequencing of

genomes of many organisms important to agriculture, industries and medicine. The human genome was sequenced by 2003 [1]. Sequencing of the entire genomes of rice, Arabidopsis and maize is going to revolutionize the complexion of plant molecular biology in the next century [10].

Laboratories in many universities and research institutions are engaged in isolation of useful genes and genetic transformation of important crop species such as cotton, rice, chickpea, sorghum and sugarcane including the introduction of insect resistant transgenic cotton by Mahyco-Monsanto and vegetable crops by Pro-Agro Corporation [10].

10. CLUSTERED REGULARLY INTER-SPACED SHORT PALINDROMIC REPEATS

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) genes (CRISPR/Cas) are essential in adaptive immunity among selected bacteria and archea enabling the organisms to respond to and eliminate invading genetic material. These repeats were initially discovered in *E. coli* and their functions were confirmed in 2007 by Barrangou and colleagues [36]. The

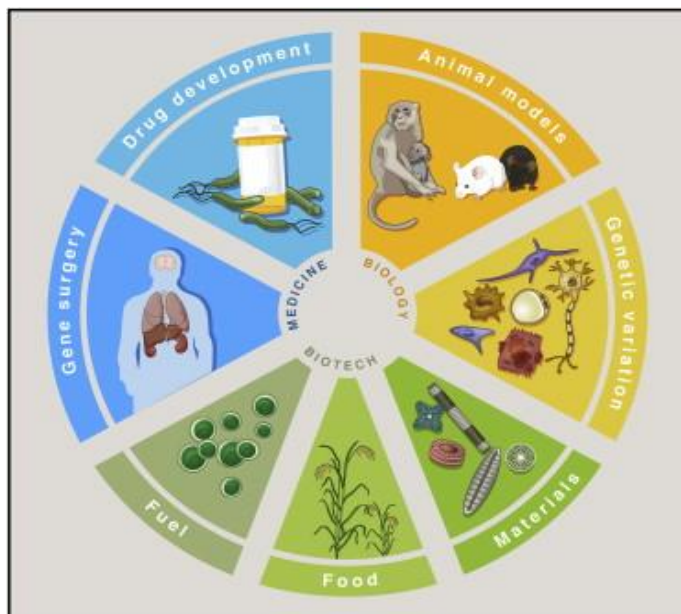


Fig. 3. Application of genetic engineering
Source: [35]

advances in genome editing technologies have overcome many challenges by allowing researchers to manipulate genomes in cell lines and animal models to more accurately model disease pathologies by using more efficient, rapid, and easy to use CRISPR/Cas system for over 20 years [37]. Cas9-mediated genome editing has enabled accelerated generation of transgenic models and expands biological research beyond traditional and genetically tractable animal model organisms. By recapitulating genetic mutations found in patient populations, CRISPR-based editing could be used to rapidly model the causal roles of specific genetic variations instead of relying on disease models that only phenocopy a particular disorder. This could be applied to develop novel transgenic animal models, to engineer isogenic ES and iPS cell disease models with specific mutations introduced or corrected, respectively, or in vivo and ex vivo gene correction [35].

Recent breakthroughs in genome engineering technology with the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) system have greatly advanced biomedical research. Its applications include rapid production of genetically modified cells in plants and animals, disease modeling, genetic corrections and inducing or repressing gene expressions.

Although its efficiency and off-target effects must be further optimized and overcome CRISPR/Cas9 is a promising gene-editing technology. Efforts have been made to use in utero electroporation-mediated CRISPR/Cas9 to study gene functions in post mitotic neurons [40].

11. MICRO RNAs TECHNOLOGY

MicroRNAs (miRNAs) are a class of small, endogenous RNAs of 21–25 nucleotides in length that plays an important regulatory role in animals and plants by targeting specific mRNAs for degradation or translation repression [38]. They are also defined as small, evolutionary conserved, single-stranded, non-coding RNA molecules that bind target mRNA to prevent protein production by one of two distinct mechanisms [39]. Mature miRNA is generated through two-step cleavage of primary miRNA (pri-miRNA), which incorporates into the effector complex RNA-induced silencing complex (RISC). The miRNA functions as a guide by base-pairing with target mRNA to negatively regulate its expression [39]. A growing body of evidence suggests that miRNAs play a role in many diverse biological processes such as development, differentiation, and apoptosis. Misregulation of miRNA expression is reported to be associated with several cancers and other diseases [41].

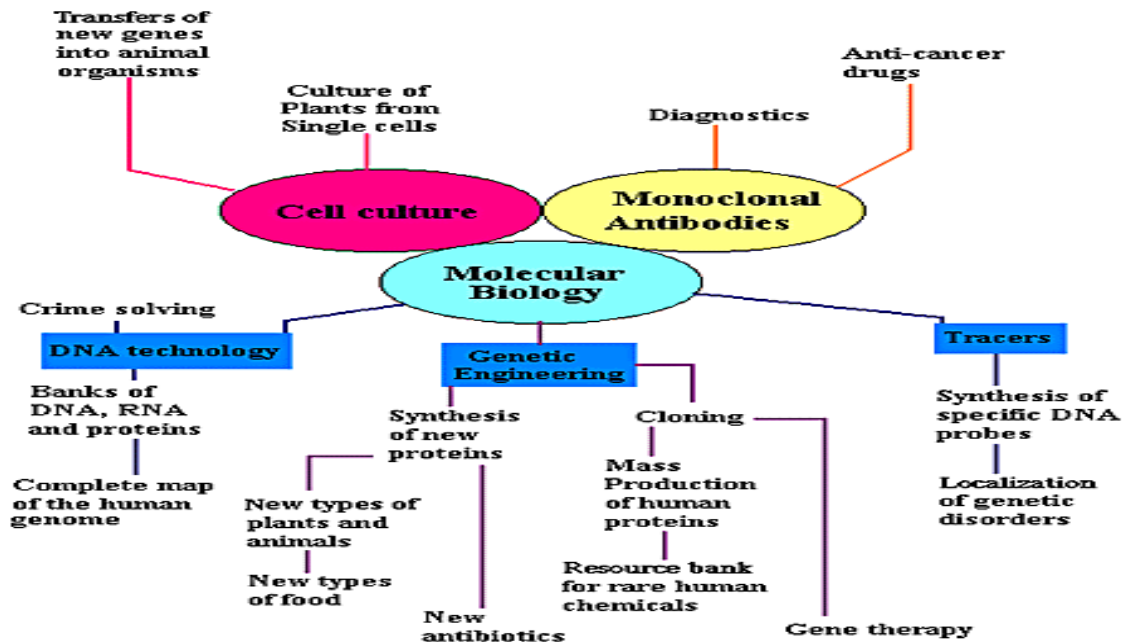


Fig. 4. Applied areas of biotechnology

Source: www.accessexcellence.org

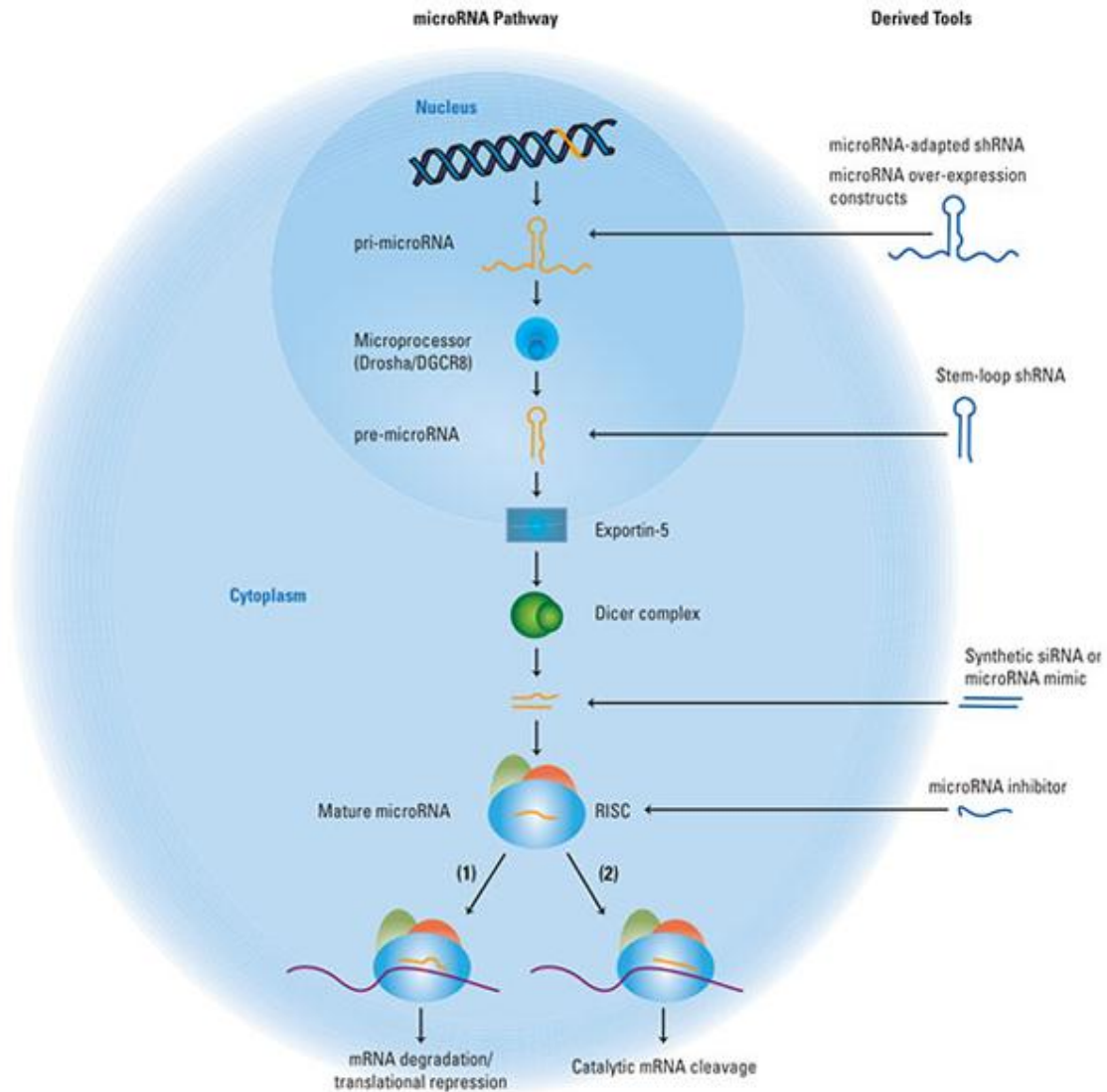


Fig. 5. A schematic of the endogenous microRNA pathway

Source: [42].

The first small RNA, *lin-4*, was discovered in 1993 by Victor and colleagues through a genetic screening in nematodes (*Caenorhabditis elegans*); later in the same year, the discovery of regulation of *lin-14* by *lin-4* has demonstrated the regulatory function of small RNAs [38,43]. In 1999, the David Baulcombe's seminal discovery that short interfering RNAs (siRNAs; approximately 21–24 nucleotides) were associated with posttranscriptional gene silencing triggered by transgenes and viruses in plants laying the conceptual foundation for groundbreaking discoveries about the RNA interference (RNAi) biochemical pathway [43].

The discovery of intergenic miRNA and protein-coding intronic miRNA coupled with work of Lee et al. in 2004 indicates that the majority of miRNA are transcribed by RNA polymerase II [39]. Following the identification of hundreds of miRNAs in various organisms, large-scale studies on miRNA expression profiles were carried out in many model organisms using northern-blot analysis, microarrays and miRNA cloning; they shows dynamic temporal and spatial expression patterns, disruption of which is associated with developmental/physiological abnormalities [44]. The regulation of miRNA function through the control of miRNA expression

or through post-transcriptional processing of events such as miRNA editing are important avenues of research that must be defined before the full potential of miRNA-based therapeutics can be realized. The research frontier in miRNA biology is now at the level of identifying new species of miRNAs, their targets and possible new functions guided by these small regulators of gene expression. Moreover, the potential role of miRNAs in modulating chromatin changes through the transcriptional gene silencing pathway is yet to be explored [45]. Without a doubt, the importance of miRNA is gaining appreciation. However, miRNA diagnosis or therapy may be many years away from entering the clinic as complex challenges remain. It should be noted that any major leap forward in miRNA research over the past decade was the result of multidisciplinary collaborations of researchers with extensive expertise in molecular biology techniques, high-throughput genomics and bioinformatics. These productive collaborations should be expended even further. With clinicians joining the club, miRNA research will be given a fresh perspective that may lead to steady progress in development of clinical applications [46].

12. CONCLUSION

Biology, Chemistry, Biochemistry and Information Technology has become an integral part of biotechnology that accelerates the production of biomolecules and bioproducts using methods that were previously not feasible or virtually thought to be impossible. Hence, biotechnological advancement has greatly improved human lives from primitive to modern and advance level.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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