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GENETIC BLUEPRINTS AND BIOCHEMICAL MACHINERY: BIOTECHNOLOGY'S ROLE IN MODERN GENETICS

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Abstract

The field of genetics has undergone substantial shifts due to the combined activities of genetic programs and chemical processes, largely informed by advancements in biotechnology. This progression has evolved from simple genetic manipulation to groundbreaking innovations across various domains. Central to this evolution is the intricate interplay between the genetic maps conveyed by DNA sequences and the chemical dynamics governing gene regulation and function. Focusing on technological advancements with significant implications in biotechnology, notable developments include gene editing tools such as the CRISPR-Cas9 system, high-throughput DNA sequencing platforms, and RNA-based therapies. Recent advances have significantly improved our ability to handle and analyze genetic data. Biotechnology is crucial in areas like gene therapy and personalized medicine, which tailor treatments to an individual's genetic profile. As biotechnology continues to progress, it will enhance our understanding of genetics, refine gene-editing techniques, and lead to new medical, agricultural, and environmental innovations. The future potential of biotechnology in genetics is vast and promising.

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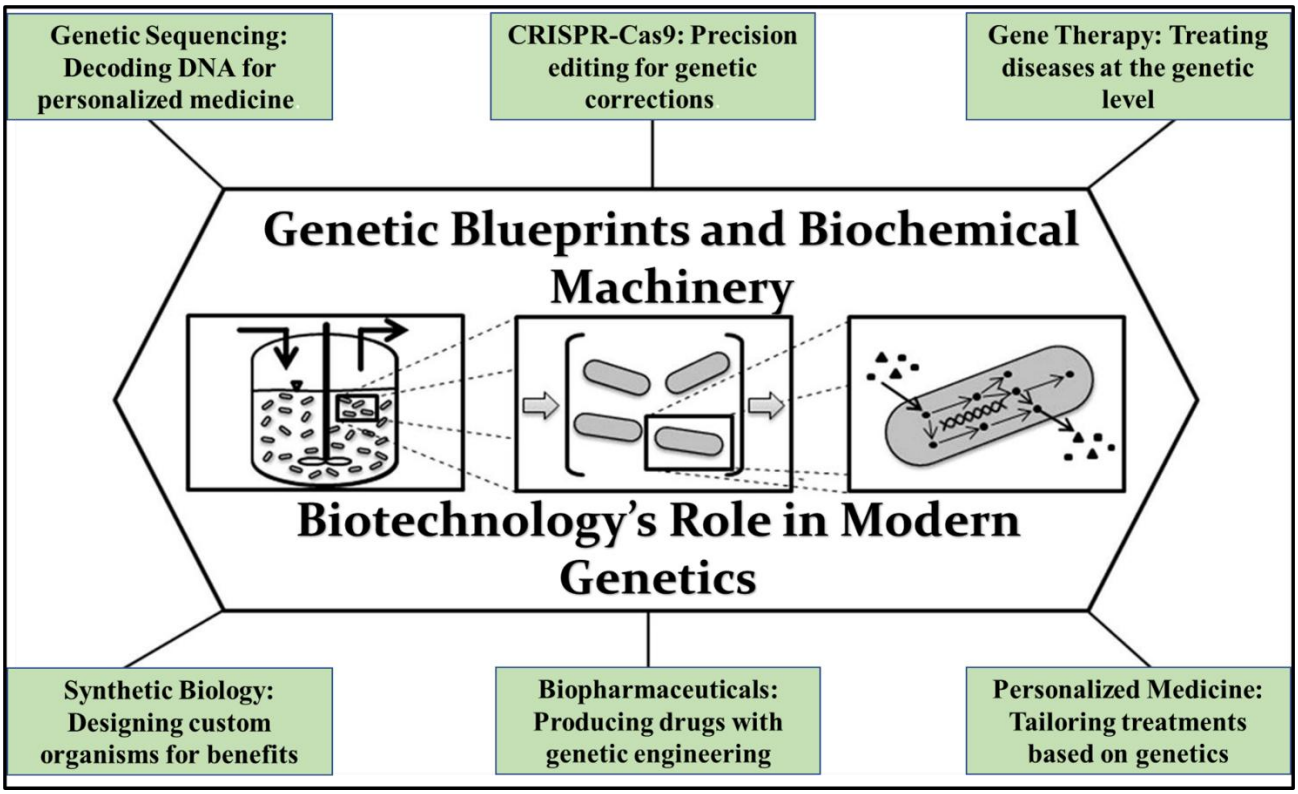

Graphical abstract

Introduction

The study of genetics has been scientifically revolutionized in the last few decades due to the advancement of biotechnology. The genetic map made up of DNA is the primary information base that builds the biochemical processes within a cell (Adlak et al., 2019). This has been an area of significant study; as a result, there have been major advancements and discoveries. In its defining aspect, genetics is a subdivision of biology that stems from the research of the Austrian geneticist Gregor Mendel, who conducted his work with pea plants in the 19th century (Gayon, 2016). However, the evolution of genetics started in the middle of the twentieth century when James Watson and Francis Crick presented the structure of DNA (Danylova & Komisarenko, 2020). This discovery revealed the helical structure of DNA and gave knowledge about how genetic information is stored and copied. Since then, genetics has grown immensely due to technological advancements that have enhanced more profound research into the molecular basis of heredity and variation (Moss, 2019).

Genome is defined as the complete set of genetic instructions in an organism or the genetic makeup of an organism. It is made up of DNA, which is made up of long sequences of nucleotides in a particular order (Brown, 2023). Nucleotide comprises a sugar, a phosphate group, and a nitrogenous base. These bases include adenine (A), thymine (T), cytosine (C), and guanine (G), which form the sequence that determines the genetic information of the development and functioning of living organisms (Brown, 2020). The developments in sequencing and analyzing genomes have been one of the most significant leaps in genetics as it has given a complete look at the genetics of different organisms. Biotechnology in genetics can be illustrated by the Human Genome Project, accomplished in 2003 (Moraes & Góes, 2016).

This global research project sought to analyze and depict all the genes in the human body and their functionality (Ali et al., 2019). The end of the Human Genome Project signified the start of the post-genomic era when researchers started to study the genetic aspect of diseases, discover variations, and use genetics to provide customized treatment to patients. It also helped improve other fields of genomics, including comparative genomics, functional genomics, and epigenomics. Biotechnology has been central in making such developments possible (Stenson et al., 2017). The innovations in recombinant DNA technology in the 1970s enabled researchers to work on genetic material in previously impossible ways. This technology involves the integration of DNA from different sources to produce new sequences and allow the analysis and manipulation of genes (Lek et al., 2016). Molecular biology methods like gene cloning, PCR, and DNA sequencing have made working with specific genes and their products easier by amplifying, analyzing, or mutating particular DNA segments (Li et al., 2018).

The CRISPR-Cas9 system is one of the most groundbreaking biotechnological inventions in genetic engineering, as it is an efficient gene editing tool that can be utilized to modify the genome. Initially identified in bacteria and involved in the immune system of the bacteria, CRISPR-Cas9 has been employed in many organisms, including human beings (Tyagi et al., 2020). This technology consists of applying a guide RNA to take the Cas9 enzyme to a particular site on the genome, thus creating a double-stranded break (V. Singh et al., 2017). CRISPR-Cas9 has introduced new ways of experimentation in genetic research, ranging from genetically modified animal models for diseases affecting human beings to the generation of gene therapies for genetic diseases. Another significant advancement of biotechnology in contemporary genetics is the development of high-throughput sequencing technologies, or next-generation sequencing (NGS) (Hossain, 2021). Next-generation sequencing has allowed researchers to perform GWAS to find diseases' genetic markers and analyze gene expression and population differences (Nasykhova et al., 2019). The availability of large volumes of genetic data has also promoted the creation of bioinformatics tools and approaches to work with it. Biotechnology and genetic engineering have expanded the knowledge of the genetic basis of inheritance and implemented many applications in different fields. It has been applied in agriculture to produce genetically modified foods with favorable characteristics like resistance to pests, diseases,

drought, and higher nutritional value (Black et al., 2015). These could increase yield, decrease reliance on chemicals, and solve problems of food insecurity when implemented (Alonso et al., 2015).

In medicine, biotechnology has improved the diagnosis and treatment of diseases. Diagnosis through genetic testing and screening has become the most efficient way of identifying patients at risk of inherited diseases so they can be treated early and efficiently (Splinter et al., 2018). Gene therapy, which involves replacing, modifying, or adding genes in a patient's body, holds promise for treating various genetic diseases. New techniques are more accurate, making treatments more effective. Personalized medicine, which tailors treatment based on an individual's genetic profile, is another important area of biotechnology and genetics. Both gene therapy and personalized medicine offer new ways to tackle diseases with greater precision." (Fernandez-Marmiesse et al., 2018).

Another area where biotechnology contributes a lot to genetics is in the synthetic biology discipline that merges engineering and biology paradigms. In this context, synthetic biology is defined as designing and constructing novel biological components, devices, and systems and modifying existing biological systems for specific uses (Goyal et al., 2022). With the help of this field, one can develop new biological products and processes that can dramatically change various sectors such as pharmaceutics, biofuels, etc. Ethical and safety issues are also observed in synthetic biology, which requires effective regulation of innovation (Madhavan & Mustafa, 2023). When exploring modern genetics technologies, it is essential to discuss the ethical, social, and consequential impacts of various biotechnology breakthroughs (Heams, 2015). Issues include the precision of controlling genetic material, potential side effects, genetic discrimination, and the use of human embryos. As we keep advancing in biotechnology, we must carefully consider these ethical and societal implications to ensure the benefits are used responsibly and fairly (Freemont & Kitney, 2015).

Genetic Blueprints: The Foundation of Life

Structure and Function of DNA

DNA, or deoxyribonucleic acid, can be defined as the hereditary material existing in almost all living organisms and being the basis of genetic information (Mattick & Amaral, 2023). The molecule’s well-known double helix form, discovered by James Watson and Francis Crick in 1953, has two polynucleotide chains running in opposite directions and coiled around a central axis (Bretscher & Mitchison, 2017). These strands are made up of repeating units called nucleotides, each consisting of three components (Haidri et al., 2023): A phosphate group, a five-carbon sugar, deoxyribose, and one of four nitrogenous bases- adenine, thymine, cytosine, and guanine. The specifics of the two base pairings (for example, A-T and C-G) are that they create hydrogen bonds, which contribute to the stability of the DNA molecule. It permits the exact copying of genetic information (M. Tripathi et al., 2023).

The sequence of these nucleotide pairs contains the information required for all living organisms' growth, development, and functioning (Haidri, Ishfaq, et al., 2024). They are segments of DNA called genes, which are used in synthesizing RNA molecules with the help of transcription (Haidri, Qasim, et al., 2024). These RNA molecules, especially mRNA, play a role in transmitting genetic information from the DNA pool in the nucleus to the ribosomes in the cytoplasm for translation. Proteins are involved in many processes essential for the organism's life, they catalyze metabolic processes, replicate DNA, react to stimuli, and transport molecules (Javed et al., 2021). The study of DNA as hereditary material has been central to genetics and has helped explain how inherited information is stored and used (Ullah et al., 2024). The discovery of the double helix structure led to other scientific discoveries, such as molecular biology techniques like DNA sequencing, PCR, and genetic engineering (Hussain et al., 2024). These techniques have significantly transformed how scientists can analyze and edit genetic material, immensely impacting the medical, agriculture, and biotechnology fields (A. Singh et al., 2021).

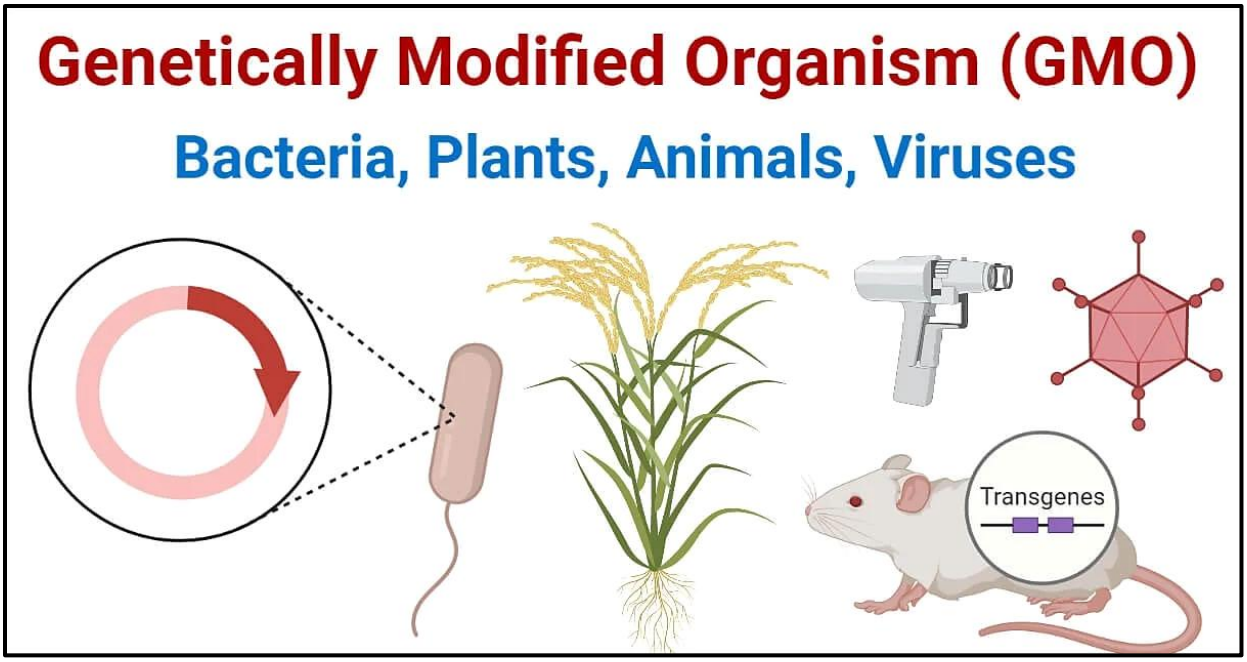

Figure 1 Genetically modified organisms

The features of DNA sequencing have allowed individuals to discover the genes relevant to certain illnesses, comprehend the genetic variation among populations, and design treatment plans (Fatima et al., 2024). Biotechnology has produced genetically modified organisms (GMOs) with desirable characteristics like high yield and disease resistance (Riaz et al., 2023). DNA has also helped to reveal the history of evolution between different animals, giving molecular support to the theory of evolution. The current study of DNA formation and functioning proves

that the study of genetics is a dynamic field that provides the basis for advancement in the life sciences (Riaz et al., 2021).

Gene Expression and Regulation

Gene expression is the information in a gene that is used to make a functional product (Hill et al., 2021). DNA carries genetic information, which is copied into messenger RNA (mRNA) using the enzyme RNA polymerase (Silverman et al., 2020). This involves opening the DNA double helix and using one strand to create an RNA molecule that matches the other strand. The mRNA sequence copies the gene's coding sequence and is used to make proteins. After it is made, the mRNA undergoes several changes, like capping the 5' end, splicing out introns, and adding a poly-A tail to the 3' end. These modifications stabilize the mRNA and help move it from the nucleus to the cytoplasm. In the cytoplasm, the ribosomes read the mRNA sequence and translate it into a specific sequence of amino acids to form a protein (Consortium, 2017).

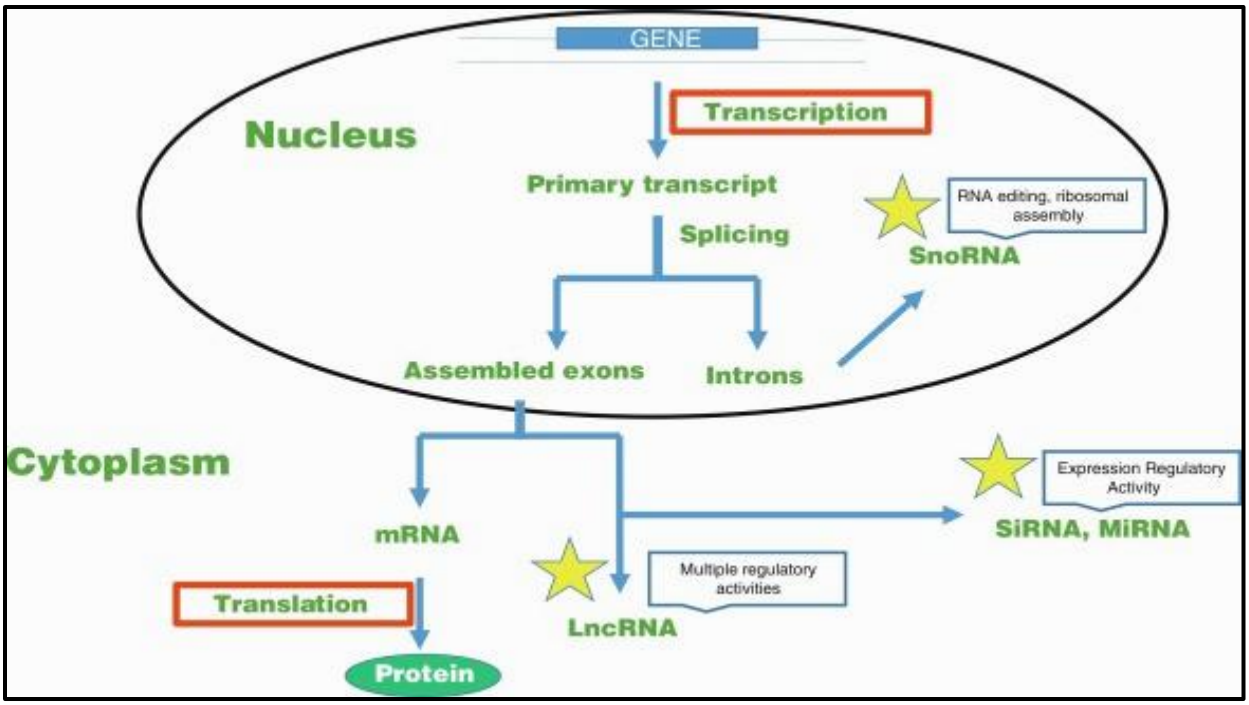

Figure.2 From Gene to Protein: The Central Dogma of Molecular Biology

Protein synthesis involves transferring RNA (tRNA) molecules that bring the correct amino acids to the sequence encoded by the mRNA codons using the tRNA's anticodon sequence (Haidri, Fatima, et al., 2024). The ribosome helps link the amino acids by forming peptide bonds, creating a growing polypeptide chain that folds into a functional protein. Gene regulation controls when, where, and how much of a gene product is made. This regulation is essential for a cell's activities, growth, and response to its environment. There are different levels of regulation: transcriptional, post-transcriptional, translational, and post-translational (Ummer et al., 2023). At

the chromosomal level, transcription factors can attach to DNA sequences near genes, either helping or blocking RNA polymerase from starting transcription. Enhancers and silencers are DNA regions that can increase or decrease transcription levels when specific transcription factors bind to them (Gil & Ulitsky, 2020). Epigenetic modifications, like DNA methylation and histone modification, also regulate gene expression by changing chromatin structure and DNA accessibility. Post-transcriptional regulation happens after mRNA is created, involving processes like mRNA splicing, editing, and stability control. Alternative splicing allows different proteins to be produced from a single gene by including different exons in the final mRNA (Corbett, 2018). mRNA stability and degradation are controlled by RNA-binding proteins and microRNAs, which interact with mRNAs to affect their lifespan and translation rate (Feigerlovà & Battaglia-Hsu, 2017). Some regulatory elements located in the mRNA can influence the binding of the ribosomes and the start of the translation. Another level of regulation of protein function is post-translational modification of the proteins produced from the synthesized mRNA, which can include phosphorylation, ubiquitination, and glycosylation to change the activity, stability, location, and interactions with other molecules. Such changes enable cells to respond quickly to environmental changes by activating or inactivating specific proteins (Feigerlovà & Battaglia-Hsu, 2017). Gene regulation is a complex process that dictates where, when, and how much of any given gene is produced to create functional organisms. Knowledge of gene expression and regulation patterns aids in decoding the cell's functions and has vast applications in developmental biology, disease investigations, and biotechnology, among others (Feigerlovà & Battaglia-Hsu, 2017).

Biochemical Machinery: The Workhorses of the Cell

Enzymes and Proteins

Enzymes are proteins that act as catalysts, speeding up biochemical reactions without being used up. They are involved in many cellular processes like DNA synthesis, repair, transcription, and translation (Kornberg, 1991). Enzymes lower the activation energy needed for a reaction, thus increasing the reaction rate. They form enzyme-substrate complexes by binding to the substrate at the active site, helping to convert substrates into products through methods like induced fit and transition state stabilization (Shanmugam, 2009). For example, during DNA replication, enzymes like DNA polymerase add nucleotides to create new DNA strands using a template strand. Helicase separates the DNA strands, primase makes RNA primers to start replication, and ligase joins breaks in the DNA to ensure continuity (Anita et al., 2024). In DNA repair, some proteins include DNA ligase, endonucleases, and exonucleases, which are responsible for identifying the errors or damage in the DNA and repairing them to ensure that the genetic material is not compromised. RNA polymerase catalyzes to convert RNA from a DNA template during transcription (Fossel, 2015). There are also proteins known as transcription factors that help initiate and control transcription so that the genes to be expressed are expressed at the right time. Likewise, enzymes such as ribonuclease and spliceosome complexes contribute to the maturation

of RNA molecules in preparation for translation to protein products. Proteins, the gene products, assume various functions within the cell, as bounded by their structure and characteristics. Connective tissue proteins like collagen and keratins help support and rigidify the tissue and cells to give strength and structure (Sindhu et al., 2022).

Table.1 Therapeutic Enzyme Applications for Various Diseases and Conditions

Disease/Condition	**Cause/Pathology**	**Therapeutic Enzymes [Brand]**	**Ref.**
α-Mannosidosis	Deficiency of α-D-mannosidase	Velmanase α [Lamzede]	Mehta & Beck (2020)
Batten disease	Deficiency of tripeptidyl peptidase 1	Cerliponase α [Brineura]	Schulz et al. (2013)
Pompe's disease	Deficiency of acid α-glucosidase	α-glucosidase [Myozyme]	Kishnani & Howell (2004)
Metabolic Deficiencies			
Exocrine pancreatic insufficiency (EPI)	Insufficient pancreatic enzymes	Pancreatic enzymes [Enzepi]	Borowitz & Stevens (2012)
Phenylketonuria (PKU)	Deficiency of phenylalanine hydroxylase (PAH)	PAH and phenylalanine ammonia-lyase [Palynziq]	Blau & van Spronsen (2010)
Severe combined immunodeficiency (SCID)	Deficiency of adenosine deaminase (ADA)	Polyethylene glycol-conjugated ADA	Hershfield (1995)
Wolman disease	Deficiency of lysosomal acid lipase	Lysosomal acid lipase [Kanuma]	Pisciotta & Busnelli (2017)
Acute intermittent porphyria (AIP)	Deficiency of hydroxymethylbilane synthase	Hydroxymethylbilane synthase and porphobilinogen deaminase	Anderson & Sassa (2006)
Congenital sucrase-isomaltase deficiency (CSID)	Deficiency of sucrase and isomaltase	Sacrosidase	Treem (1995)
Hypophosphatasia	Deficiency of alkaline phosphatase (TNSALP)	TNSALP [Strensiq]	Whyte & Greenberg (2012)
Protein C deficiency	Deficiency of Protein	Protein C [Ceprotin]	Griffin & Evatt

	C		(1992)
Lactose intolerance	Reduction of lactase activity	Lactase	Woteki & Thomas (1998)
Fibrosis Conditions			
Chronic total occlusions	Fibrous plaques in coronary arteries	Collagenase Clostridium histolyticum (CCH)	de Oliveira & Silveira (2014)
Dupuytren's disease	Thickening of fascia tissue in hands	Collagenase Clostridium histolyticum (CCH) [Xiapex]	Badalamente & Hurst (2007)
Peyronie's disease	Fibrous plaques in the penis	Collagenase Clostridium histolyticum (CCH)	Gelbard & Jarow (2007)
Uterine fibroid	Fibroid tissue growth around the uterus	Collagenase Clostridium histolyticum (CCH)	Ulbrich & von Rappard (2010)
Keloid disease	Overgrowth of scar tissue	Collagenases and matrix metallopeptidases	Bloemen & van der Wal (2009)
Lung cystic fibrosis	Viscous secretions in lungs	Deoxyribonuclease I [Pulmozyme]	Yankaskas & Marshall (2004)
Glaucoma	Fibrous formations at trabecular meshwork	Collagenases	Coleman & Migdal (2008)
Ocular Affections			
Various ocular diseases	Malfunction of vitreous humor	Chondroitinase, hyaluronidase, nattokinase, ocriplasmin [Jetrea]	Avery (2009)

The cytoskeleton, composed of actin and tubulin, is essential for cell movement, division, and transport (Aseervatham, 2020). Proteins like hormones and receptors help cells and tissues communicate and change their functions. For example, insulin helps glucose enter cells, and neurotransmitter receptors manage signals between neurons. Signaling pathways involve interactions between proteins and phosphorylation, where kinases add phosphate groups to regulate actions (Biswas et al., 2022). Metabolic pathways are vital for cell survival, and enzymes drive these processes by breaking down nutrients like carbohydrates, fats, and proteins for energy and other uses. Amylase breaks down starch into sugars, lipase breaks down fats into

fatty acids and glycerol, and proteases break down proteins into amino acids (Pizzagalli et al., 2021). Transport proteins, such as hemoglobin and membrane transporters, are crucial for moving molecules and ions within and between cells. Hemoglobin carries oxygen from the lungs to tissues, while membrane transporters move nutrients into cells and waste out (Cherian, 2022). Proteins also play a role in the immune response, with antibodies identifying and neutralizing pathogens. Regulatory proteins control gene expression, cell division, and cell death (Nair, 2016).

Proteins have diverse functions due to their structures, which are determined by genes and amino acid sequences. They form specific shapes through interactions like hydrogen bonds and ionic bonds. Changes or mutations in proteins can lead to diseases like cystic fibrosis, Alzheimer's, and cancer. Understanding enzymes and proteins is crucial for understanding cell processes and molecular organization. This knowledge has significantly advanced medicine, biotechnology, and bioengineering, leading to enzyme therapy, drug design, and improved biomaterials (Maiuri & Kroemer, 2018).

RNA and Ribosomes

RNA molecules are very crucial in the transfer of genetic information from DNA to proteins. Different RNA molecules include mRNA, tRNA, rRNA, and micro-RNA, all involved in gene expression. mRNA transports the copied code from DNA to those structures called ribosomes, where the code is manifested into proteins. tRNA transports suitable amino acids to the ribosome during translation and ensures that the appropriate amino acids are attached in sequence to the polypeptide chain, depending on the template mRNA (Catalanotto et al., 2016). The most well-known rRNA is the molecular part of ribosomes. In contrast, these particles are unique complexes that synthesize new proteins according to the information in a message, an mRNA molecule (Scherrer, 2018).

Ribosomes are large structures that are made up of rRNA and proteins. These are two subunits, the large and the small, and they combine during translation. The ribosome brings mRNA and tRNA molecules together so that the process of mRNA translation into a polypeptide chain can occur. The small subunit interacts with the mRNA, and the large subunit contributes to forming peptide linkages between the amino acids (de Farias & José, 2020). This process involves three main steps: This has been divided into three phases: initiation phase, elongation phase, and termination phase. In initiation, the ribosome comes around the target mRNA, and the first tRNA is attached to the start codon. When the process is in the elaboration phase, the ribosome translates along the mRNA to codons where the appropriate aminoacyl-tRNA will form the new polypeptide chain. In termination, the ribosome encounters a stop codon, releasing the newly synthesized protein while the ribosome falls apart (Shen et al., 2018).

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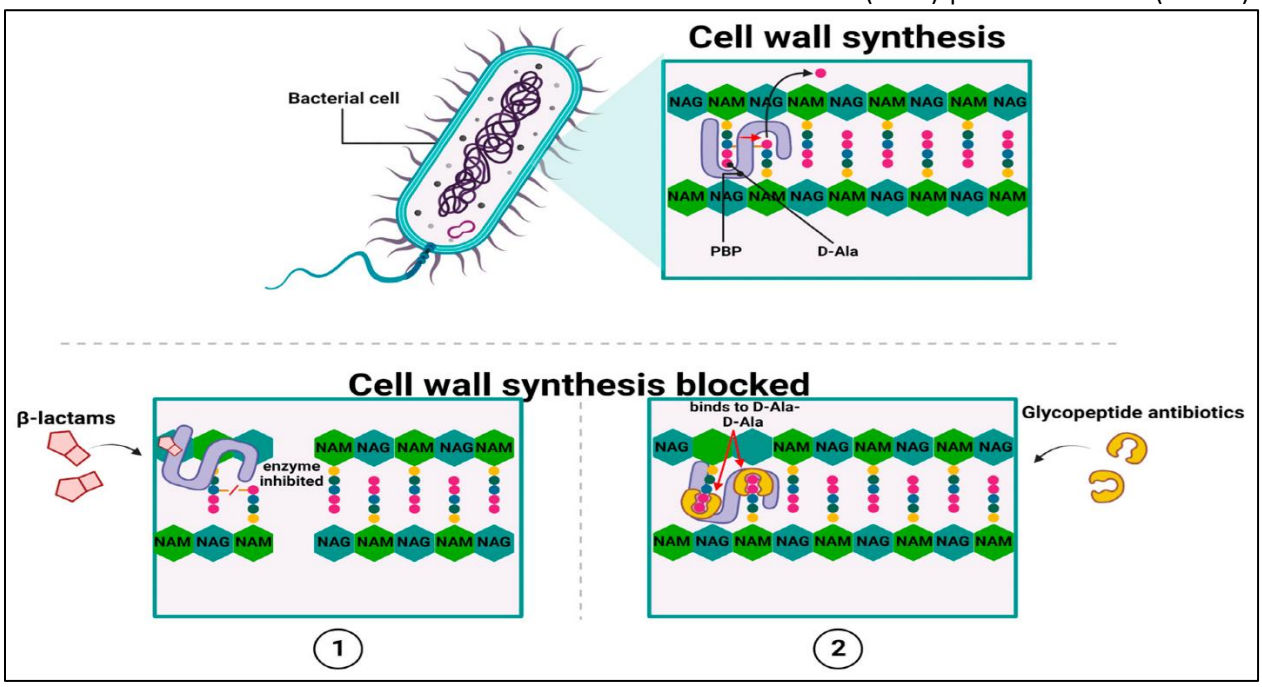

Figure3 Bacterial mechanism in the antibiotics process (Baran et al., 2023)

Recombinant DNA Technology

Recombinant DNA technology or rDNA technology uses laboratory techniques to combine and restructure DNA in an organism, thus even modifying an organism's genetic material. This field has evolved genetic research and technological applications that offer instruments for scientists to analyze and manage genetic operations as they choose. The main ideas of recombinant DNA technology are gene cloning, PCR, polymerase chain reaction, and CRISPR-Cas9 (Sivamani et al., 2024). Gene cloning is a process in which a gene of interest is placed into a vector, most commonly in the form of a plasmid, and then introduced into the host organism, often bacteria. This makes it possible to isolate and magnify the particular gene of interest to help investigate processes it controls and regulates (T. A. Singh et al., 2020). Cloning is helpful as it provides the researcher with large quantities of a gene or the protein product of the gene for use in gene therapy, the production of pharmaceuticals, and agriculture. PCR is one of the other fundamental methods that facilitate amplifying specific DNA sequences, starting with a small amount of the source material. This involves heating and cooling cycles where the DNA strands are denatured, annealed, and extended, and as such cycles are repeated, there is exponential replication of DNA. PCR has been established as one of the central technologies in molecular biology used to amplify DNA in diagnostics, forensics, and evolution studies (Bhatia & Goli, 2018).

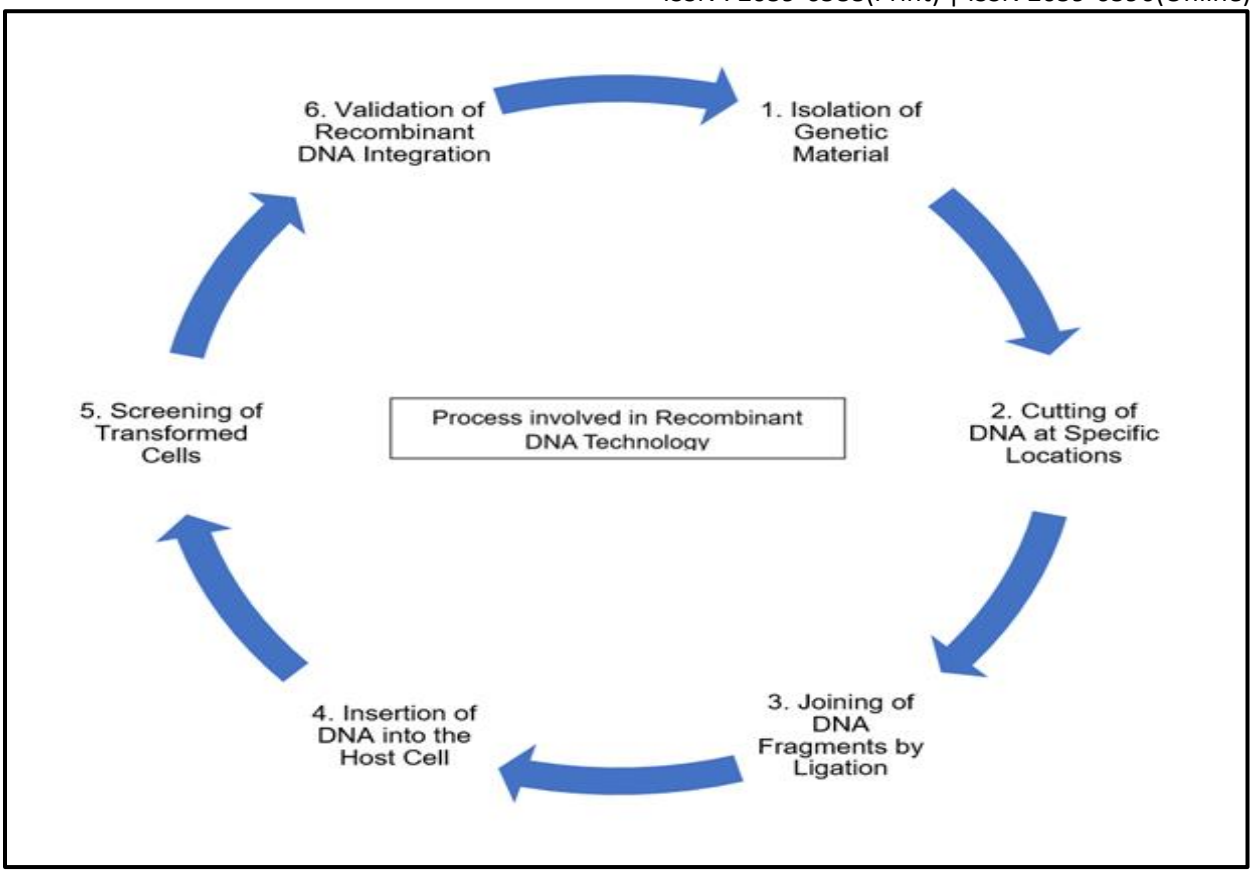

Fig 4. Process involved in recombinant DNA technology

CRISPR-Cas9 is another widely innovative development in recombinant DNA technology that came later. A gene-editing tool, CRISPR-Cas9, is a bacterial immune system that can efficiently target and cut DNA sequences (Tüzmen et al., 2018). A guide RNA recognizes the target DNA sequence and can guide the Cas9 endonuclease to specific loci within the genome to create double-strand breaks. This allows for particular manipulations, for example, gene knockouts, insertions, or corrections, which has transformed the analysis of gene function and the creation of gene therapies for genetic diseases. The application aspect is not limited to research; medicine, agriculture, and industry are some areas that have been affected by recombinant DNA technology (Puvanakrishnan et al., 2019).

Table.2 Genome-Scale Metabolic Models of Industrial Platform Strains

Organism	**Main Products/Applications**	**Model**	**Description**	**Reference**
E. coli K-12 MG1655	Biofuel, multipurpose recombinant proteins	iML1515	1515 genes, 2719 reactions, 1192 metabolites	Lee & Kim, 2020

S. cerevisiae	Alcoholic beverages, bakery products, bioethanol	Yeast8, ecYeast8, panYeast8, coreYeast8, proYeast8	1133 genes, 3949 reactions, 2680 metabolites	Sandberg et al., 2019
C. glutamicum ATCC13032	Amino acids	iCW773	773 genes, 1207 reactions, 950 metabolites	Ohno et al., 2017
B. subtilis	Industrial enzymes and antibiotics	iBsu1144	1144 genes, 1955 reactions, 1103 metabolites	Pfeifer et al., 2017
Alternaria sp. MG1	Resveratrol	iYL1539	1539 genes, 2255 reactions, 2231 metabolites	McCloskey et al., 2018
S. coelicolor	Antibiotics, secondary metabolites	Sco-GEM, EcSco-GEM	1777 genes, 2612 reactions, 2073 metabolites	Ajikumar et al., 2010
C. vulgaris	Lipids, pigments for biofuel, food supplements	iCZ946	946 genes, 2294 reactions, 1770 metabolites	Kumar & Singh, 2017
L. mesenteroides subsp. cremoris ATCC 19254	Starter in food fermentation (dairy, meat, vegetable products)	iLM.c559	559 genes, 1088 reactions, 1129 metabolites	Lee et al., 2011
L. reuteri JCM 1112	Starter in food fermentation, probiotic products, reuterin	Lreuteri_530	530 genes, 710 reactions, 658 metabolites	Michalak & Brigham, 2015
N. salina	Lipids, pigments for biofuel, food supplements	iNS934	934 genes, 2345 reactions, 1985 metabolites	Lee et al., 2018
C. reinhardtii	Biofuel	nd*	3726 reactions, 2436 metabolites	Sandberg et al., 2020

In medicine, this technology has led to new treatments, such as recombinant insulin and other therapeutic proteins, and advancements in gene therapy to fix genetic disorders. In agriculture, scientists have created genetically modified crops with better traits, like resistance to pests, diseases, and drought, as well as improved nutritional value. These crops help feed the world and support sustainable farming (Arjmand et al., 2020). Recombinant DNA technology also allows us to make biofuels, bioplastics, and other products using specially engineered microorganisms. As the field develops, we expect to discover more methods and applications that will address global challenges and improve people's lives (Bhoria et al., 2022).

Gene Editing and CRISPR-Cas9

CRISPR-Cas9 is a powerful and precise tool for editing genes that has transformed genetic research and biotechnology. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, and Cas9 is the enzyme that acts like a molecular scissor guided by RNA (Shah et al., 2018). The guide RNA finds a specific DNA sequence in the genome, and then the Cas9 enzyme cuts that exact spot. This cut allows scientists to add, delete, or replace genetic material, enabling targeted changes to the genome. CRISPR-Cas9 has many uses (Akram et al., 2023). It helps researchers study gene functions more effectively by creating specific gene knockouts or mutations. This has increased our understanding of gene regulation, development, and genetic diseases. Additionally, CRISPR-Cas9 is used in functional genomics to perform high-throughput screening, helping to identify genes that control various cellular processes and diseases (Wang et al., 2018).

Table 3 Gene Editing in Various Disease Models: Target Genes, Functions, and Methods

Disease/Disorder	Model/System	Target Gene(s)	Gene Function	Editing Method	References
Rheumatoid Arthritis (RA)	RAW264 Cells	miR-155	Pro-inflammatory regulation in RA	Knockout	Jing et al., 2015
Hereditary Tyrosinemia Type 1 (HT-1)	Mouse	FAH	Tyrosine catabolism pathway, toxic accumulation	Gene editing	Yin et al., 2016
Mice	Hpd	Gene disruption	Pankowicz et al., 2016		
Ornithine Transcarbamylase (OTC)	Mice	OTC	Urea cycle involvement	Gene editing	Yang et al., 2016

Deficiency					
Arginase Deficiency	iPSC Cells	Arg1	Final step in urea cycle control	Gene knock-in	Lee et al., 2016a
Duchenne Muscular Dystrophy (DMD)	Mouse	Dmd	Dystrophin protein truncation	Gene addition/deletion	Zhu et al., 2017; Li et al., 2015a
Skeletal Muscle Stem Cells				El Refaey et al., 2017	
Limb Girdle Muscular Dystrophy Type 2B (LGMD2B)	Mice, iPSC Stem Cells	DYSF, α-sarcoglycan	Dysferlin production stabilises dystrophin	Gene correction	Turan et al., 2016
Diabetes Mellitus Type 1 (DM1)	Mouse	DMPK	Disease aetiology	Gene editing	van Agtmaal et al., 2017
Huntington's Disease (HD)	Fibroblasts, Mouse	HTT	Brain function regulation; huntingtin synthesis	Gene editing, ORF deletion	Monteys et al., 2017; Talan, 2015
Friedreich Ataxia (FA)	Mouse	Frataxin	Mitochondrial oxidative stress involvement	Gene editing	Ouellet et al., 2017
Amyotrophic Lateral Sclerosis (ALS)	iPSC Cells	SOD1, FUS	RNA/DNA binding proteins	Gene editing	Wang et al., 2017
Cystic Fibrosis (CF)	iPSC Cells	CFTR	Chlorine transport regulation	Base editing	Firth et al., 2015
Alpha-1 Antitrypsin Deficiency (AATD)	iPSC Cells	AAT	Serum trypsin inhibitor	Gene disruption	Smith et al., 2015
Recessive Dystrophic Epidermolysis	Mice	COL7A1	Collagen production	Gene disruption	Hainzl et al., 2017

Bullosa (RDEB)					
Dominant Dystrophic Epidermolysis Bullosa (DDEB)	iPSC Cells	COL7A1	Collagen production	Mutant allele disruption	Shinkuma et al., 2016
Multidrug Resistance in Infectious Diseases	Escherichia coli, Galleria mellonella	ft, and, msbA, nusB, ease, blaSHV-18, blaNDM1, gyrA, NDM-1, CTX-M-15, blaTEM, blaSHV, aph-3, mecA	Cell division control, bacterial population, drug resistance	Gene knockout/deletion, disruption	Gomaa et al., 2014; Citorik et al., 2014; Yosef et al., 2015; Kim et al.
Acquired Immunodeficiency Syndrome (AIDS)	CHME5 Cells, Mice	LTR U3 region, gag, pol	Viral transcriptional activity control	Knockout	Hu et al., 2014; Yin et al., 2017a; Bella et al., 2018
Burkitt's Lymphoma, Hodgkin's Disease	SNU-719	BART5, BART6	Capsid protein expression control	Gene deletion	van Diemen et al., 2016
Herpes Simplex Virus (HSV) Infection	TC620 Cells	ICP0, ICP4, ICP27	Capsid protein expression control	Indels	Roehm et al., 2016

In therapeutic approaches, the potential of CRISPR-Cas9 can be described as enormous in treating genetic diseases. With this technology, it is possible to treat diseases such as cystic fibrosis, sickle cell anemia, and muscular dystrophy, permanently since the mutations are

corrected at the DNA level (Abdelnour et al., 2021). Clinical trials are currently being conducted for CRISPR-based treatments, and the initial findings are quite encouraging. Also, the use of CRISPR-Cas9 in agriculture, for instance, is to modify crops to have better yield, disease-resistant crops, and crops that can be resistant to drought (Bhattacharjee et al., 2022). It is also used in synthetic biology and designing genetically modified organisms to manufacture biofuels, drugs, and other valuable bioproducts. CRISPR-Cas9 is easy to use, can target any gene, and has been made available globally for laboratories, boosting innovation in many areas. As researchers improve this method and sort out ethical and safety issues, CRISPR-Cas9 is set to be one of the leading technologies in genetic engineering that can bring changes to medicine, agriculture, and biotechnology that are expected to transform the world (Luthra et al., 2021).

Genomics and Bioinformatics

High sequencing technologies have transformed genomics, the study of entire genomes. These technologies allow scientists to sequence and compare large amounts of genetic data quickly and affordably (R. Tripathi et al., 2016). Next-generation sequencing (NGS) tools help scientists sequence entire genomes from humans to microorganisms, enhancing our understanding of genetic structures, functions, and variations (Raza & Ahmad, 2019). Genomics has several areas, including whole-genome sequencing, genome-wide association studies (GWAS), and comparative genomics. Genome sequencing helps identify genetic differences such as SNPs, insertions, deletions, and structural variations that are linked to diseases (Nazipova et al., 2018). GWAS uses extensive genomic data to find connections between genetic markers and traits, improving our knowledge of genetic risks and supporting personalized medicine (Pereira et al., 2020).

Bioinformatics is a fast-growing field in biology and medicine that involves organizing and analyzing genomic data. It uses computational tools and methods to analyze and visualize data, predict gene functions, and study species' evolutionary relationships. Bioinformatics applications include sequence identification, gene expression analysis, protein folding, and gene function prediction. Tools and databases like BLAST, Ensembl, and UCSC Genome Browser are commonly used for these purposes (Javed et al., 2021). Bioinformatics also integrates genomics with other fields like transcriptomics, proteomics, and metabolomics, providing deeper insights into biological processes and disease mechanisms (Ari & Arikan, 2016).

Applications of Biotechnology in Modern Genetics

Genetic Engineering and GMOs

Biotechnology has enhanced the field of genetic engineering, which creates new GMOs with various favorable characteristics to solve diverse issues in agriculture and food production. Genetic engineering is a method of direct intervention in the organism’s genome through technology to insert, augment, or alter particular genes. This process generally involves

techniques like recombinant DNA technology, gene editing, and transformation to induce the intended genetic changes (Abdul Aziz et al., 2022). Genetic engineering is widely used to produce genetically modified crops, also called bio crops. Techniques like genetic engineering allow crops with specific characteristics, including pest resistance, herbicide tolerance, and enhanced nutritional qualities (Javed et al., 2021). For example, Bt cotton and Bt corn are genetically modified to produce proteins from the bacterium *Bacillus thuringiensis*, which is deadly to certain insects but harmless to man and animals. This trait helps cut the use of chemical pesticides, benefiting the environment and the wallet. Likewise, crop plants with herbicide tolerance allow farmers to use herbicides to control weeds, increasing the yield and decreasing the cost of agriculture (Yali, 2022).

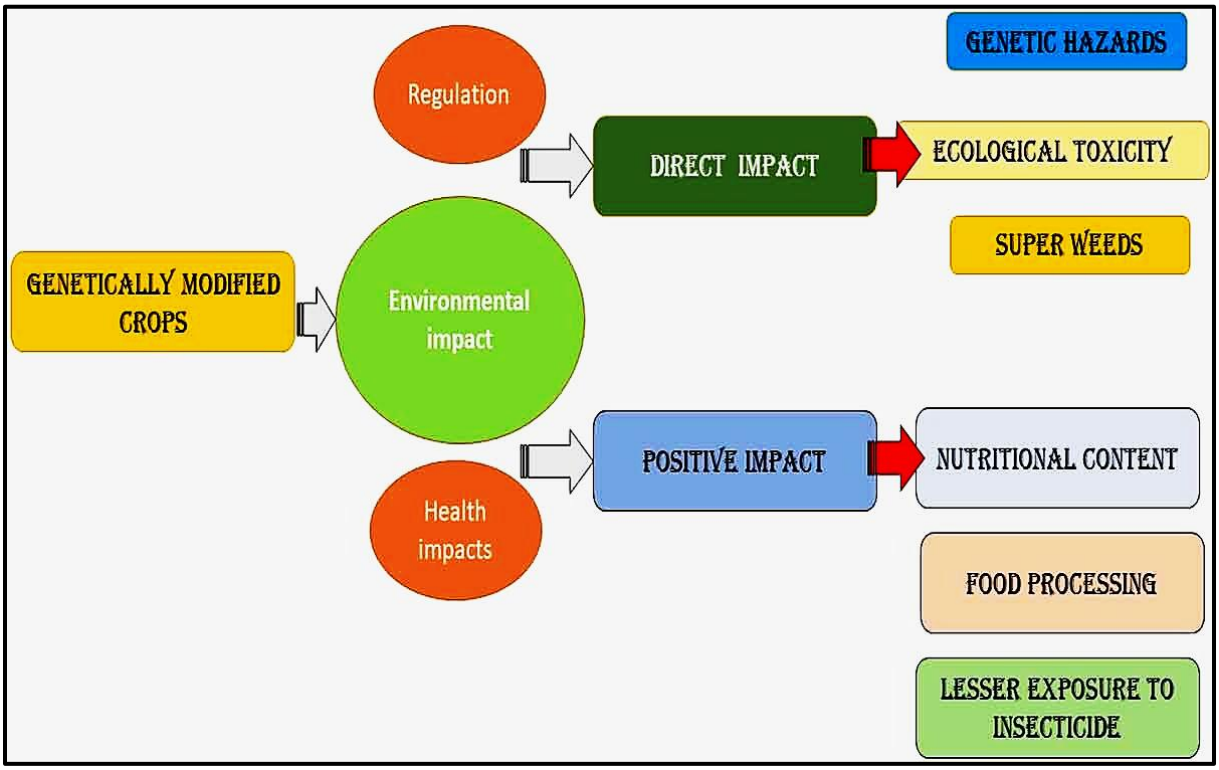

Figure 5. Genetically modified crops are more resilient to environmental impacts

Another improvement is that foods' nutritional value has been improved. Some transgenic crops like Golden Rice have been bioengineered to contain higher provitamin A, or beta-carotene, to help eliminate provitamin A deficiency in developing countries. Such modifications can significantly influence public health since the quality of staple foods eaten by a vast population is enhanced. Some of the effects of genetic engineering and GMOs are not limited to agriculture only (Javed et al., 2021). Besides improving the ability of crops to withstand biotic and abiotic stresses and enhancing their nutritional value, such technologies can be helpful in the management of the physical environment and sustainable practices (Pereira et al., 2020). For instance, biotechnological applications such as genetically modified microorganisms can be used

in bioremediation to help remove substances that pollute the environment. These microorganisms can break down pollutants better than other microorganisms. Moreover, it is applied in synthesizing drugs and industrial enzymes, enhancing the production of medicine and biotechnology (Napier et al., 2019).

Table4. Comprehensive Overview of Gene Editing Techniques for Disease Resistance and Nutritional Enhancement in Agriculture and Livestock

Trait Category	Target Trait	Plant/Animal Species	Gene Editing Technique	Reference(s)
Disease Resistance	Broad-spectrum Disease Resistance	Barley	CRISPR/Cas9	Borrelli et al. (2018)
Disease Resistance	Phytophthora Resistance	Cacao	CRISPR/Cas9	Fister et al. (2018), Zeng et al. (2020)
Disease Resistance	Potato Virus Y	Potato	CRISPR/Cas9	Butler et al. (2016), Butler et al. (2017)
Disease Resistance	Bacterial Blight Resistance	Rice	CRISPR/Cas9	Oliva et al. (2019), Blanvillain-Baufumé et al. (2017)
Disease Resistance	Bacterial Blight	Rice	TALENs	Li et al. (2012)
Disease Resistance	Bacterial Blight	Rice	CRISPR/Cas9	Xu et al. (2019)
Disease Resistance	Powdery Mildew	Wheat	CRISPR/Cas9, TALENs	Wang et al. (2014)
Disease Resistance	Mastitis	Cattle	ZFN	Proudfoot et al. (2015), Proudfoot et al. (2016)
Disease Resistance	Bacterial Speck	Tomato	CRISPR/Cas9	Nekrasov et al. (2017), Pyott et al. (2016)
Disease Resistance	Banana Streak	Banana	CRISPR/Cas9	Tripathi et al. (2019)
Nutritional	Reduced Starch	Cassava	CRISPR/Cas9	Zorrilla-López et

Enhancement				al. (2013), Veley et al. (2013)
Nutritional Enhancement	Reduced Phytate Levels	Maise	ZFN	Liang et al. (2014)
Nutritional Enhancement	Reduced Phytic Acid	Maize	TALENs, CRISPR/Cas9	Shukla et al. (2009)
Nutritional Enhancement	Increased Oleic Acid Content	Peanut	TALENs	Al Amin et al. (2019)
Nutritional Enhancement	Reduced Starch	Potato	CRISPR/Cas9	Clasen et al. (2015)
Nutritional Enhancement	Prevented Cadmium Uptake	Rice	CRISPR/Cas9	Tang et al. (2017)
Nutritional Enhancement	Increased Carotenoids	Rice	CRISPR/Cas9	Dong et al. (2020)
Nutritional Enhancement	Low Gluten for Reduced Allergenicity	Wheat	CRISPR/Cas9	Sánchez-León et al. (2018)
Nutritional Enhancement	Increased Beta-Carotene	Banana	CRISPR/Cas9	Kaur et al. (2018)
Nutritional Enhancement	Reduced Linoleic and Linolenic Acid	Brassica napus	CRISPR/Cas9	Okuzaki et al. (2018)

Therapeutic Interventions

Gene therapy is an advanced treatment that aims to correct or prevent inherited diseases by introducing new genetic material into the patient's body. This method involves adding, removing, or changing genes to fix defective ones that cause diseases (Arjmand et al., 2020). Gene therapy targets the disease's root cause, which was often not possible in the past. Biotechnology has improved gene therapy, especially in delivery systems. One common technique uses viral vectors to transfer therapeutic genes into target cells (Sayed et al., 2022). These vectors are designed to be safe and effective, helping deliver new genes into cells or producing therapeutic proteins inside them. For instance, adeno-associated viruses and lentiviruses are frequently used vectors that have succeeded in clinical trials for various genetic disorders (Oggu et al., 2017).

Table. 5 Overview of Gene Therapy Products: Target Diseases, Viral Vectors, and Transgenes

Product Name	Targeted Disease	Origin Virus	Introduced Gene	Replication Capability	Source
Rexin-G	Various Solid Tumors	Gamma-retrovirus	Cyclin G1	Non-replicating	Chawla et al., 2019
Strimvelis	ADA-SCID	Gamma-retrovirus	ADA	Non-replicating	Cicalese et al., 2018
Zalmoxis	Leukemia	Gamma-retrovirus	HSV-thymidine kinase	Non-replicating	Vago et al., 2012
Yescarta	Diffuse Large B-cell lymphoma	Gamma-retrovirus	Chimeric T Cell Receptor	Non-replicating	Neelapu et al., 2017
Invossa	Osteoarthritis	Gamma-retrovirus	TGF-β1	Non-replicating	Cherian et al., 2015
Kymriah	B-Cell Acute Lymphoblastic Leukemia	Lentivirus	Chimeric T-Cell Receptor	Non-replicating	Maude et al., 2018
Zynteglo	Beta-thalassemia Requiring Transfusions	Lentivirus	β-globin	Non-replicating	Thompson et al., 2018
Gendicine	Head and Neck Squamous Cell Carcinoma	Adenovirus 5	p53	Non-replicating	Normile, 2018a
Oncorine (H101)	Nasopharyngeal Carcinoma	Adenovirus 5	None	Replicating	van Dijke et al., 2018
Imlygic	Melanoma Not Amenable to Surgery	Herpes Simplex Virus	GM-CSF	Replicating	Collichio et al., 2018
Glybera	Lipoprotein Lipase Deficiency	AAV1	Lipoprotein Lipase	Non-replicating	Normile, 2018b
Luxturna	Leber's Congenital Amaurosis	AAV2	RPE65	Non-replicating	Van Til et al., 2010
Zolgensma	Spinal	AAV9	SMN1	Non-	Shahryari et

	Muscular Atrophy Type I			replicating	al., 2019

Personalized Medicine

The use of genetic information in clinical practice has led to the development of individualized medicine, a new approach to delivering medical treatments and interventions based on a patient's genetic makeup (Goetz & Schork, 2018). This is a new concept in the field of medicine that focuses on improving the accuracy and effectiveness of medical treatments about genetics that may define a patient's response to medications, diseases' prevalence, and general well-being (Hassan et al., 2022). Personalized medicine utilizes genomics and bioinformatics to incorporate information beyond the conventional "standard" treatment method. In pharmacogenomics, an individual's genetic variation is compared with known disease genes to determine the genetic variation of the inheritance of diseases, the metabolism of drugs, and the response to treatment (Seyhan & Carini, 2019). This helps to personalize the treatment programs closer to a patient's genetic makeup, thereby enhancing the efficacy of the therapeutic interventions and reducing the likelihood of side effects (Schirrmacher, 2019).

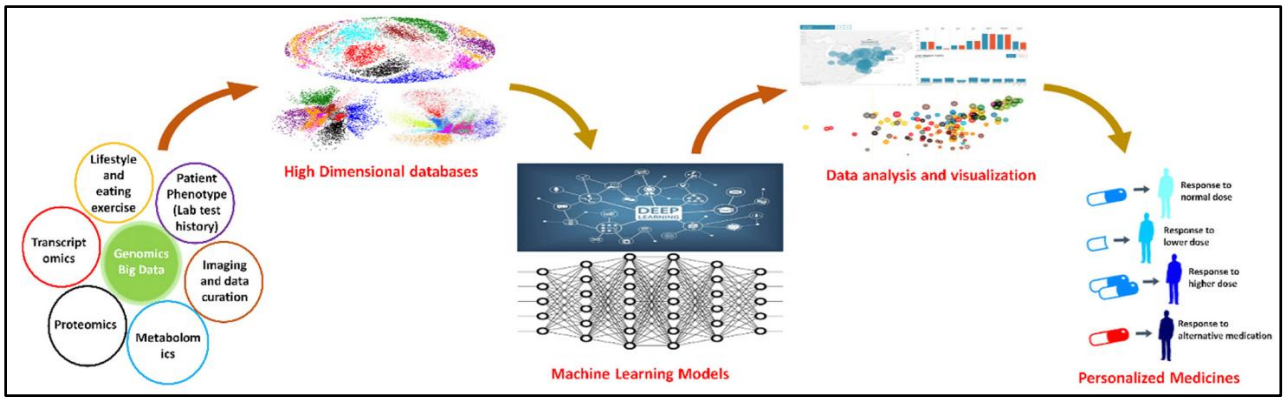

Figure 6. The computational approach for personalized medicine.

An excellent example of the use of personalized medicine is in the treatment of cancer, where the genetic makeup of tumors is screened to identify the genes that cause the growth of cancer cells (Mansouri et al., 2020). This information can help choose specific treatments aimed for particular changes within the cancer cells, increasing the effectiveness of the therapy and reducing the impact on the rest of the body. It also applies to prevention and health promotion, as genetic data can evaluate an individual's likelihood of developing a specific disease and take the appropriate prevention measures (Zhang et al., 2018). For instance, genetic risk factors can be used to recommend changes in people's behavior and preventive measures to address potential diseases like cardiovascular diseases and diabetes (Goetz & Schork, 2018).

Table 6. Comparison of Whole Genome Sequencing, Whole Exome Sequencing, and Target Panel Techniques

Technique	Coverage	Variants Detected	Advantages	Limitations	References
WGS	Entire genome	~4,000,000	- Detects all genome variants - Identifies genome rearrangements and structural variants - Uniform depth of sequencing	- Highest cost - Largest data volume - Long, complex analysis - Limited clinical application	van Dijk et al., 2018; Goodwin et al., 2016; Shendure & Ji, 2008; Koboldt et al., 2013; Mardis, 2017
WES	2% of genome	~20,000	- Targets all protein-coding regions - Lower cost than WGS	- Incomplete exome coverage - Cannot detect non-coding and structural variants - Requires exome capture or enrichment	Clark et al., 2011; Mertes et al., 2011; Shendure & Ji, 2008
Target Panels	Specific genes	Variable	- Customizable - Lowest cost - Rapid analysis - Suitable for clinical applications	- Limited to selected genes - Limited novel and structural variant detection - Needs updates with new discoveries	Mertes et al., 2011; Ku et al.,

Future Prospects

Biotechnology in genetics is a rapidly growing field full of exciting possibilities. Innovations and new topics keep emerging, making this study area very promising. Several areas will shape the

future of genetic research and its applications, potentially making significant changes in medicine and agriculture (Straathof et al., 2019). One of the most groundbreaking advances is the improvement of gene editing tools. While CRISPR-Cas9 is already famous for genetic manipulation, new versions like CRISPR/Cas12 and CRISPR/Cas13 are being developed for better precision and fewer unintended effects. New techniques such as base editing and prime editing offer even more accurate ways to make genetic changes without causing double-strand breaks, which helps avoid unwanted mutations. These advances could lead to a better understanding and treatment of genetic diseases and allow for producing organisms with desired traits (Belizário & Napolitano, 2015).

Conclusion

Biotechnology has revolutionized our approach to genetics and the study of DNA. It provides powerful tools and techniques that improve how we explore and manipulate genetic material. With methods like CRISPR-Cas9 gene editing, scientists can make precise changes to DNA, offering new ways to treat genetic disorders. Techniques such as next-generation sequencing allow for detailed analysis of genomes, helping researchers understand genetic variations and their impacts. Gene therapy can correct defective genes that cause diseases, while RNA-based therapies can regulate gene activity to treat various conditions. Personalized medicine uses a person's genetic information to tailor treatments, making them more effective and reducing side effects. However, as these technologies advance, it is crucial to consider their ethical implications, including privacy concerns and the potential for genetic discrimination. Establishing clear ethical guidelines and fostering discussions about the responsible use of biotechnology is essential for maximizing benefits and minimizing risks.

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