

RECOMBINANT DNA TECHNOLOGY AND ITS APPLICATIONS: A REVIEW**Shivangi Singh*, Dr. Rohit Mohan, Dr. Amit K. Srivastava, Dr. Amresh Gupta, Dr. Pritt Verma and Neelam Verma**

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ABSTRACT

Biotechnology which is synonymous with genetic engineering or recombinant DNA (rDNA) is an industrial process that uses the scientific research on DNA for practical applications. rDNA is a form of artificial DNA that is made through the combination or insertion of one or more DNA strands. It offered new opportunities for innovations to produce a wide range of therapeutic products with immediate effect in the medical genetics and biomedicine by modifying microorganisms, animals, and plants to yield medically useful substances. Recombinant DNA technology is playing a vital role in improving health conditions by developing new vaccines and pharmaceuticals. This review gives brief introduction to rDNA and its applications in various fields.

KEYWORDS: Chimeric DNA, restriction enzymes, Transgenic Plants, Gene Therapy.**INTRODUCTION**

The advances in recombinant DNA technology have occurred in parallel with the development of genetic processes and biological variations. The development of new technologies has resulted into production of large amount of biochemically defined proteins of medical significance and created an enormous potential for pharmaceutical industries. The biochemically derived therapeutics is large extra cellular proteins for use in either chronic replacement therapies or for the treatment of life-threatening indications.^[1,2] Human life is greatly affected by three factors: deficiency of food, health problems, and environmental issues. Food and health are basic human requirements beside a clean and safe environment. With increasing world's population at a greater rate, human requirements for food are rapidly increasing. Humans require safe-food at reasonable price. Several human related health issues across the globe cause large number of deaths. Approximately 36 million people die each year from noncommunicable and communicable diseases, such as cardiovascular diseases, cancer, diabetes, AIDS/HIV, tuberculosis, malaria. Despite extensive efforts being made, the current world food production is much lower than human requirements, and health facilities are even below standard in the third-world countries. Rapid increase in industrialization has soared up the environmental pollution and industrial wastes are directly allowed to mix with water, which has affected aquatic marines and, indirectly, human-beings. Therefore, these issues urge to be addressed through modern technologies. Unlike tradition approaches to overcome agriculture, health, and environmental issues through breeding, traditional medicines, and pollutants

degradation through conventional techniques respectively, the genetic engineering utilizes modern tools and approaches, such as molecular cloning and transformation, which are less time consuming and yield more reliable products. For example, compared to conventional breeding that transfers a large number of both specific and nonspecific genes to the recipient, genetic engineering only transfers a small block of desired genes to the target through various approaches, such as biolistic and Agrobacterium-mediated transformation.^[3] The alteration into plant genomes is brought either by homologous recombination dependent gene targeting or by nuclease-mediated site-specific genome modification. Recombinase mediated site-specific genome integration and oligonucleotide directed mutagenesis can also be used.^[4]

Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. Recombinant DNA was first achieved in 1973 Herbert Boyer, of the University of California at San Francisco, and Stanley Cohen, at Stanford University, who used *E. coli* restriction enzymes to insert foreign DNA into plasmids.^[5] Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure. Recombinant DNA molecules are sometimes

called chimeric DNA, because they can be made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends. The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms. Proteins that can result from the expression of recombinant DNA within living cells are termed recombinant proteins. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced.^[6] Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences.^[7] Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

Advantages of Recombinant technology

- Provide substantial quantity
- No need for natural or organic factors
- Tailor made product that you can control
- Unlimited utilizations
- Cheap
- Resistant to natural inhibitors

Disadvantages of Recombinant technology

- Commercialized and became big source of income for businessmen
- Effects natural immune system of the body
- Can destroy natural ecosystem that relies on organic cycle
- Prone to cause mutation that could have harmful effects
- Major international concern: manufacturing of biological weapons such as botulism & anthrax to target humans with specific genotype
- Concerns of creating super-human race

The Basics of Recombinant DNA

That's a very good question! rDNA stands for recombinant DNA. Before we get to the "r" part, we need to understand DNA. Those of you with a background in biology probably know about DNA, but a lot of Chem's haven't seen DNA since high school biology. DNA is the keeper of the all the information needed to recreate an organism. All DNA is made up of a base consisting of sugar, phosphate and one nitrogen base. There are four nitrogen bases, adenine (A), thymine (T), guanine (G),

and cytosine (C). The nitrogen bases are found in pairs, with A & T and G & C paired together. The sequence of the nitrogen bases can be arranged in an infinite way, and their structure is known as the famous "double helix" which is shown in the image below. The sugar used in DNA is deoxyribose. The four nitrogen bases are the same for all organisms. The sequence and number of bases is what creates diversity. DNA does not actually make the organism; it only makes proteins. The DNA is transcribed into mRNA and mRNA is translated into protein, and the protein then forms the organism. By changing the DNA sequence, the way in which the protein is formed changes. This leads to either a different protein, or an inactive protein. Now that we know what DNA is, this is where the recombinant comes in. Recombinant DNA is the general name for taking a piece of one DNA, and combining it with another strand of DNA. Thus, the name recombinant, Recombinant DNA is also sometimes referred to as "chimera." By combining two or more different strands of DNA, scientists are able to create a new strand of DNA. The most common recombinant process involves combining the DNA of two different organisms.^[8]

How is Recombinant DNA made?

There are three different methods by which Recombinant DNA is made. They are Transformation, Phage Introduction, and Non-Bacterial Transformation. Each are described separately below. Transformation The first step in transformation is to select a piece of DNA to be inserted into a vector. The second step is to cut that piece of DNA with a restriction enzyme and then ligate the DNA insert into the vector with DNA Ligase. The insert contains a selectable marker which allows for identification of recombinant molecules. An antibiotic marker is often used so a host cell without a vector dies when exposed to a certain antibiotic, and the host with the vector will live because it is resistant.

The vector is inserted into a host cell, in a process called transformation. One example of a possible host cell is E. Coli. The host cells must be specially prepared to take up the foreign DNA. Selectable markers can be for antibiotic resistance, color changes, or any other characteristic which can distinguish transformed hosts from untransformed hosts Different vectors have different properties to make them suitable to different applications. Some properties can include symmetrical cloning sites, size, and high copy number. Non-Bacterial Transformation This is a process very similar to Transformation, which was described above. The only difference between the two is non-bacterial does not use bacteria such as E. Coli for the host. In microinjection, the DNA is injected directly into the nucleus of the cell being transformed. In biolistic, the host cells are bombarded with high velocity microprojectiles, such as particles of gold or tungsten that have been coated with DNA. Phage Introduction Phage introduction is the process of transfection, which is equivalent to transformation, except a phage is used instead of

bacteria. In vitro packaging of a vector is used. This uses lambda or M13 phages to produce phage plaques which contain recombinants. The recombinants that are created can be identified by differences in the recombinants and non-recombinants using various selection methods.

Working of rDNA

Recombinant DNA works when the host cell expresses protein from the recombinant genes. A significant amount of recombinant protein will not be produced by the host unless expression factors are added. Protein expression depends upon the gene being surrounded by a collection of signals which provide instructions for the transcription and translation of the gene by the cell. These signals include the promoter, the ribosome binding site, and the terminator. Expression vectors, in which the foreign DNA is inserted, contain these signals. Signals are species specific. In the case of *E. Coli*, these signals must be *E. Coli* signals as *E. Coli* is unlikely to understand the signals of human promoters and terminators. Problems are encountered if the gene contains introns or contains signals which act as terminators to a bacterial host. This results in premature termination, and the recombinant protein may not be processed correctly, be folded correctly, or may even be degraded. Production of recombinant proteins in eukaryotic systems generally takes place in yeast and filamentous fungi. The use of animal cells is difficult due to the fact that many need a solid support surface, unlike bacteria, and have complex growth needs. However, some proteins are too complex to be produced in bacterium, so eukaryotic cells must be used.

Importance of rDNA

Recombinant DNA has been gaining in importance over the last few years, and recombinant DNA will only become more important in the 21st century as genetic diseases become more prevalent and agricultural area is reduced. Below are some of the areas where Recombinant DNA will have an impact.

- Better Crops (drought & heat resistance)
- Recombinant Vaccines (Hepatitis B)
- Prevention and cure of sickle cell anemia
- Prevention and cure of cystic fibrosis
- Production of clotting factors
- Production of insulin
- Production of recombinant pharmaceuticals
- Plants that produce their own insecticides
- Germ line and somatic gene therapy.^[9]

Application of recombinant DNA technology^[10]

1. Production of Transgenic Plants: By utilizing the tools and techniques of genetic engineering it is possible to produce transgenic plants or the genetically modified plants. Many transgenic plants have been developed with better qualities like resistance to herbicides, insects or viruses or with expression of male sterility etc.

2. Production of Transgenic Animals: By the use of rec DNA technology, desired genes can be inserted into

the animal so as to produce the transgenic animal. The method of rec DNA technology aids the animal breeders to increase the speed and range of selective breeding in case of animals. It helps for the production of better farm animals so as to ensure more commercial benefits. Another commercially important use of transgenic animals is the production of certain proteins and pharmaceutical compounds. Transgenic animals also contribute for studying the gene functions in different animal species. Biotechnologists have successfully produced transgenic pigs, sheep, rats and cattle.

3. Production of Hormones: By the advent of techniques of rec DNA technology, bacterial cells like *E. coli* are utilized for the production of different fine chemicals like insulin, somatostatin, somatotropin and p-endorphin. Human Insulin Hormone i.e., Humulin is the first therapeutic product which was produced by the application of rec DNA technology.

4. Production of Vaccines: Vaccines are the chemical preparations containing a pathogen in attenuated (or weakened) or inactive state that may be given to human beings or animals to confer immunity to infection. A number of vaccines have been synthesized biologically through rec DNA technology, these vaccines are effective against numerous serious diseases caused by bacteria, viruses or protozoa. These include vaccines for polio, malaria, cholera, hepatitis, rabies, smallpox, etc. The generation of DNA vaccines has revolutionized the approach of treatment of infectious diseases. DNA-vaccine is the preparation that contains a gene encoding an immunogenic protein from the concerned pathogen.

5. Biosynthesis of Interferon: Interferon's are the glycoproteins which are produced in very minute amounts by the virus-infected cells. Interferon's have antiviral and even anti-cancerous properties. By recombinant DNA technology method, the gene of human fibroblasts (which produce interferons in human beings) is inserted into the bacterial plasmid. These genetically engineered bacteria are cloned and cultured so that the gene is expressed and the interferons are produced in fairly high quantities. This interferon, so produced, is then extracted and purified.

6. Production of Antibiotics: Antibiotics produced by microorganisms are very effective against different viral, bacterial or protozoan diseases. Some important antibiotics are tetracycline, penicillin, streptomycin, novobiocin, bacitracin, etc. recombinant DNA technology helps in increasing the production of antibiotics by improving the microbial strains through modification of genetic characteristics.

7. Production of Commercially Important Chemicals: Various commercially important chemicals can be produced more efficiently by utilizing the methods of rec DNA technology. A few of them are the alcohols and alcoholic beverages obtained through fermentation;

organic acids like citric acid, acetic acid, etc. and vitamins produced by microorganisms.

8. Application in Enzyme Engineering: As we know that the enzymes are encoded by genes, so if there are changes in a gene then definitely the enzyme structure also changes. Enzyme engineering utilizes the same fact and can be explained as the modification of an enzyme structure by inducing alterations in the genes which encode for that particular enzyme.

9. Prevention and Diagnosis of Diseases: Genetic engineering methods and techniques have greatly solved the problem of conventional methods for diagnosis of diseases. It also provides methods for the prevention of a number of diseases like AIDS, cholera, etc. Monoclonal antibodies are useful tools for disease diagnosis. Monoclonal antibodies are produced by using the technique called hybridoma technology.

10. Gene Therapy: Gene therapy is undoubtedly the most beneficial area of genetic engineering for human beings. It involves delivery of specific genes into human body to correct the diseases. Thus, it is the treatment of diseases by transfer and expression of a gene into the patients' cells so as to ensure the restoration of a normal cellular activity.

11. Practical Applications of Genetic Engineering: recombinant DNA technology has an immense scope in Research and Experimental studies.

It is applied for:

- a. Localizing specific genes.
- b. Sequencing of DNA or genes.
- c. Study of mechanism of gene regulation.
- d. Molecular analysis of various diseases.
- e. Study of mutations in DNA, etc.

12. Applications in forensic science: The applications of rec DNA technology (or genetic engineering) in forensic sciences largely depend on the technique called DNA profiling or DNA fingerprinting. It enables us to identify any person by analyzing his hair roots Wood stains, serum, etc. DNA fingerprinting also helps to solve the problems of parentage and to identify the criminals.

13. Biofuel Production: Biofuels are derived from biomass and these are renewable and cost effective. Genetic engineering plays an essentially important role in a beneficial and largescale production of biofuels like biogas. bio hydrogen biodiesel bio-ethanol, etc. Genetic engineering helps to improve organisms for obtaining higher product yields and product tolerance.

14. Genetically stable high producing microorganisms are being developed by using modern recombinant DNA techniques, which aid in an efficient production of bioenergy.

15. The energy crop plants are those plants which use solar energy in a better way for production of biomass. Genetic improvements of these energy crop plants greatly help for quick and high Product on of biomass which in turn reduces the biofuel production cost. The fermenting microbes which are utilized for biogas production are improved at the genetic level for achieving better result.

16. Environment Protection: Genetic engineering makes its contributions to the environment protection in various ways. Most important to mention are the new approaches utilized for waste treatments and bioremediation Environment protection means the conservation of resources and hence to limit the degradation of environment.

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