



Recombinant DNA Technology: Molecular Construction of Genes for Biological Production

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DESCRIPTION

Recombinant Deoxyribonucleic Acid (DNA) technology is a molecular biotechnology approach used to combine genetic material from different biological sources and introduce the resulting DNA construct into a suitable host cell. This technology allows specific genes to be isolated, modified, transferred and expressed under controlled biological conditions. Its applications include production of therapeutic proteins, vaccines, enzymes, diagnostic reagents, agricultural products and numerous industrial biomolecules. The method depends on the ability to manipulate DNA precisely and use living cells as systems for copying or expressing selected genetic information.

DNA contains the hereditary information required for the development and functioning of living organisms. A particular segment of DNA, commonly referred to as a gene, can contain instructions for producing a protein or functional Ribonucleic Acid (RNA) molecule. Recombinant DNA technology makes it possible to obtain a selected gene from one source and combine it with DNA from another source. The resulting recombinant molecule can then be introduced into a host organism where the inserted genetic sequence may be replicated or expressed. The process generally begins with identification and isolation of the desired genetic sequence. DNA can be obtained from cells, tissues, microorganisms, or other biological materials. Restriction enzymes are frequently used to cut DNA at specific recognition sequences. These enzymes generate DNA fragments with defined ends, allowing selected fragments to be joined with compatible DNA molecules. DNA ligase can then connect the fragments by forming phosphodiester bonds within the DNA backbone.

A vector is commonly used to carry the selected gene into a host cell. Plasmids are widely used vectors because they are small, circular DNA molecules that can replicate independently within suitable bacterial cells. A plasmid used for recombinant DNA work may contain an origin of replication, a selectable marker

and a region where foreign DNA can be inserted. Some vectors also contain regulatory sequences that control expression of the inserted gene. After the desired DNA fragment has been inserted into a vector, the recombinant molecule can be introduced into a host cell. Bacterial cells such as *Escherichia coli* are frequently used because they can grow rapidly and are comparatively easy to manipulate. Other hosts, including yeast, insect cells and mammalian cells, may be selected when the target protein requires particular cellular processing or structural modification.

Selection is required to identify cells that have received the recombinant DNA. Selectable markers present in vectors can provide resistance to particular antibiotics or allow cells to grow under defined conditions. Cells carrying the vector can therefore be distinguished from cells that did not receive it. Further molecular methods can confirm whether the desired DNA sequence is present and correctly positioned within the vector. Gene expression depends on regulatory sequences surrounding the inserted DNA. A promoter provides a site where transcription begins, while additional regulatory elements can influence the amount and timing of gene expression. When a recombinant gene is expressed successfully, the host cell produces the corresponding RNA and, when appropriate, the encoded protein. The amount of protein produced depends on the host system, vector design, gene sequence, culture conditions and regulatory elements.

Recombinant DNA technology has had major importance in pharmaceutical biotechnology. Human insulin is one of the well-known products produced using genetically engineered microorganisms. Before recombinant production became widely available, insulin was obtained from animal pancreatic tissue. Microbial production allowed human insulin to be manufactured using genetically modified cells, providing a consistent biological source. Recombinant systems are also used to produce growth hormones, clotting factors, therapeutic enzymes and other medical proteins. Vaccinology has also benefited from recombinant DNA methods. Genetic sequences

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encoding selected antigens can be introduced into suitable expression systems to produce purified vaccine components. Recombinant hepatitis B vaccine is an important example in which a viral antigen is produced using genetically modified yeast cells. Such approaches allow specific components to be manufactured without requiring production of the complete infectious organism.

Industrial biotechnology uses recombinant DNA technology to improve microbial production of enzymes and other valuable compounds. Genes encoding amylases, proteases, lipases, cellulases and specialty enzymes can be introduced into suitable hosts. Increasing the expression of selected genes can improve

enzyme production and simplify manufacturing. Recombinant microorganisms can also be designed to produce vitamins, amino acids, organic acids and other biochemical products. Agricultural biotechnology is another application area. Genes associated with selected traits can be introduced into plant cells through suitable genetic transformation systems. Certain genetically modified crops have been developed to provide insect resistance, herbicide tolerance, altered nutritional characteristics, or improved environmental performance. The use of such crops is subject to regulatory requirements that differ among countries.