

DEALING WITH DNA IN MOLECULAR LEVEL

Introduction

The science of genetics has entered a new era with the introduction of new techniques to deal with genetic material in molecular level. From 1970 onwards, the use of recombinant DNA technology or genetic engineering has been introduced to produce novel life forms not found in nature. Through this technology, it has been possible to transfer genes from mammals into bacteria, causing the microbes to become factories for making in large quantities, proteins of great economic importance such as hormones. The production of these proteins by conventional methods, by extraction and purification has been very expensive thus restricting their use in medicine and in economically important other activities.

Besides, attempts are now being made to transfer genes from bacteria to plants and alteration of existing genes by genetic manipulations to construct new plant varieties with desired qualities such as improved grain quality, resistance to diseases and increased photosynthetic efficiency.

Basic requirements for such manipulations are the thorough understanding of the biology, genetics, biochemistry and physiology of these organisms in order to understand precisely, the metabolic activities and key features of their regulation.

We start our discussion on genetic engineering with a brief description of major attributes of life and analysing how the genetic information and protein synthesis are interconnected, in order to produce specific features of an individual life forms which we call a "living organism".

What is life?

Life on our planet exists as an individual living systems which we call organisms. Enormous variability of different organisms makes it difficult to define the proper nature and the specificity of life. It is evident that it should contain general information on properties which are specific for all living organisms. In its generalized form, life is a specific, extremely advanced form of material evolution emerged in a particular period of its development, for which hierarchic organization is a characteristic feature. It exists in the form of discrete units (organisms) of open, self regulating systems which can utilize energy and selected substances for the production of structures with specific functions and properties under the guidance of hereditary programmes.

Major properties of life

1. Discreteness and organization (living forms exist as individual organisms which are highly organized).
2. Specific organization in different levels.
3. Cell Structure
4. Heredity and variation
5. Self replication
6. Self regulation
7. Specific interrelations with the environment
8. Growth and development
9. Irritability
10. Death

All these features and properties altogether reflect the attribute of life in its active form.

Organization levels of life forms:

Life can be studied in different levels of its organization. Molecular, macromolecular, supramolecular or sub cell, cell, tissue or organ, organism and biospheric levels.

In each level of its organization, specific features which are characteristics for the living state can be observed. For example in molecular level, structure and function of molecules which are specific for living organisms can be observed. Although the basic elements such as carbon, nitrogen, hydrogen and oxygen of which the biomolecules are composed can be found in non living things, the chemical composition of living organism is qualitatively different from that of non living things.

Cell-the structural and functional unit of living organisms

Cell is considered as the unit of biological activity in a living organism. Each cell in any organism contains hereditary information, according to which specific chemical reactions and energy transformation occur. Structural organization of the cell facilitates in providing optimal conditions for such reactions in proper time and space. As a consequence of the interaction with the environment and regulation of all metabolic processes,

internal environment of a particular cells in a stable status which we call homeostasis. All properties and characteristics of an individual organism determined by the biochemical processes take place inside the cells.

Interaction with the environmental factors

Metabolism total of all the reactions occurring in the cell

Properties and characteristics of an individual organism.

Molecular content of the cell

Chemical composition of a cell is qualitatively and quantitatively different from its environment. Intermediate metabolites and biomonomers (amino acids and nucleotides etc.) penetrating into the cell or synthesized within the cell are identical in all organisms. In certain conditions they can be appeared in nature. Nevertheless the chemical identity of living organisms are biopolymers. Specificity of these polymers, mainly the nucleic acids and proteins determine the specific properties and characteristics of each organism.

Molecular content of the cell

Inorganic molecules (mol-weight 18-44)	Intermediate metabolites & biopolymers(mol- weight 50-350)	Organic molecules Biopolymers(mol- weight 10-3-10-9)
Other inorganic molecules and water ions which are abundant even in non living matter.	Organic acids, simple sugar, amino acids mononucleotides etc.	Nucleic acids(DNA, RNA) proteins, polysaccharides

These biopolymers are capable of integrating with each other and with other chemical components of the cell to form complete organic molecules with specific functions in an organism.

Biological Polymers

Biopolymers	Biomonomers
Deoxyribo Nucleic acid (DNA)	momonudeotides (4 types of deoxyribo Nucleic acids)
Nucleic-----	-----
Acids Ribonucleic acids (RNA)	Mononucleotides (4 types of Ribonucleic acids)
Proteins	Amino Acids (20 types)
Polysaccharides	Different Monosaccharides

Major Functions of Nucleic Acids and Protein Synthesis

As it has been mentioned earlier, the nucleic acids and proteins determine the specificity of characteristic features and properties of organisms.

Nucleic acids contain hereditary information about the structure of specific proteins, involved in protein synthesis and transfer genetic information from one cell to another during cell division and reproduction.

Specific nucleic acids	Specific proteins	specific properties and characteristics of an organism.
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Nucleic acids are biopolymers consists of monomers called nucleotides.

Each nucleotide consist of a phosphoric acid, deoxyribose sugar and one of the four nitrogen bases, adenine, guanine, cytosine and thymine. Therefore, there are four different kinds of nucleotides possible in a DNA molecule depending on the nitrogen base. DNA molecule is composed of two strands bonded together in the form of double helix. In this double helix nitrogen bases of one strand preferentially bond with another base on the other strand. From four bases adenine of one strand always pair with thymine of the opposite strand and guanine of one strand pair with cytosine of the other strand.

Manupulation with genetic material

The era of molecular genetics and genetic engineering followed the discovery of DNA structure when the fundamental unit of heredity was determined to be the DNA nucleotides and the gene was found to

1. Instrumentation and techniques
2. Radioactive tracers
3. Nucleic acid enzymology

Basic methods of genetic engineering includes

1. Isolation of DNA
2. Clearance of DNA molecules into pieces by restriction endonuclease
3. Insertion of these pieces to vector molecule to clone them in an appropriate host cell.
4. Processing of cloned DNA fragments

To make recombinant DNA molecules function in vitro, it is necessary to cut the desired nucleotide sequence from the donor source and splice it into the recipient bacterial chromosome. The vehicle for transferring the donor gene into the recipient cell is a circular plasmid. The plasmid is extracted from the bacterial cell and purified. It is then cut at one position by a restriction endonuclease. The donor DNA may be derived by either of the following two methodologies. In "Shotgun" experiments, the donor DNA is unselectively broken into many pieces and attempts are made to isolate the desired protein from all of the potential recombinants. In more selective experiments, only one or a few isolated donor genes are exposed to recipient vehicles.

Shotgun Experiments

Donor DNA is isolated and cut into many pieces by the same restriction endonuclease used to cleave the plasmid. The two kinds of fragments are mixed in vitro and allowed to randomly rejoin their sticky ends to form circles. This is called a recumbinant DNA molecule. This is then inserted into a plasmid free bacterial cells and clons of cells that contain the desired recombinant plasmid and manufacture the donor protein can be identified by various techniques. (enzymatic, serological).

Selective Experiments

If the desired protein is very small and its primary structure is known, it should be possible (using genetic code information) to chemically synthesize a corresponding DNA molecule. This synthesized DNA molecule is then transferred to vectors to be cloned and to obtain proper expression. By this method it is possible to produce in large quantities desired proteins useful in medicine such as insulin and somatostatin.

DNA Sequencing Analysis

One of the most important methods in genetic engineering in the DNA Sequencing Analysis. In recombinant DNA technology it is important to know the DNA segments that do not code for Polypeptide products. These segments include promoters, operators, leader sequences and the like, which are participated in gene regulation. One of the most popular methods for rapid sequencing of relatively short DNA segments of about 100 nucleotides is that of Maxam and Gilbert.

Conclusion

Fundamental concept of recombinant DNA technology or genetic engineering is the manipulation with genes in order to construct novel organisms with desired qualities. This includes the construction of microorganisms which produce valuable proteins and novel biochemical pathways, improvement of crop varieties by increasing seed protein content, resistance to diseases and other important characteristics and production of vaccines. This can be achieved by transferring genes between different organisms and synthesizing novel genetic information by the specific alteration of cloned genes. Increased interest in exploiting methods of genetic engineering in plant science, medicine and in other areas of applied molecular biology is caused by its ability to circumvent natural barriers of incompatibility between species and generations on the one hand and highly specific manipulation of genetic material that cannot be done by conventional breeding methods on the other.