MICROBIAL GENETICS IN MIDICAL MICROBIOLOGY

Microbial Genetics: Introduction

The science of **genetics** defines and analyzes **heredity**, or constancy and change in the vast array of physiologic functions that form the properties of organisms. The basic unit of heredity is the **gene**, a segment of deoxyribonucleic acid **(DNA)** that encodes in its nucleotide sequence information for a specific physiologic property. The traditional approach to genetics has been to identify genes on the basis of their contribution to **phenotype**, or the collective structural and physiologic properties of an organism. A phenotypic property, be it eye color in humans or resistance to antibiotics in a bacterium, is generally observed at the level of the organism. The chemical basis for variation in phenotype is change in **genotype**, or alteration in the DNA sequence, within a gene or within the organization of genes.

Amplification of specific regions of DNA can be achieved with bacterial enzymes using **polymerase chain reaction (PCR)** or other enzyme-based method of nucleic acid amplification. DNA amplified by these sources and digested with appropriate **restriction enzymes** can be inserted into plasmids. Genes can be placed under control of high-expression bacterial **promoters** that allow encoded proteins to be expressed at increased levels. Bacterial genetics have fostered development of **genetic engineering** not only in prokaryotes but also in eukaryotes.

The Eukaryotic Genome

The **genome** is the total genetic information in an organism. Almost all of the eukaryotic genome is carried on two or more linear chromosomes separated from the cytoplasm within the membrane of the nucleus. **Diploid** eukaryotic cells contain two **homologues** of each chromosome. **Mutations**, or genetic changes, frequently cannot be detected in diploid cells because the contribution of one gene copy compensates for changes in the function of its homologue. A gene that does not achieve phenotypic expression in the presence of its homologue is **recessive**, whereas a gene that overrides the effect of its homologue is **dominant**. The effects of mutations can be most readily discerned in **haploid** cells, which carry only a single copy of most genes. Yeast cells (which are eukaryotic) are frequently investigated because they can be maintained and analyzed in the haploid state.

The Prokaryotic Genome

Most prokaryotic genes are carried on the bacterial chromosome. And with few exceptions, bacterial genes are haploid. Genome sequence data from more than 340 microbial genomes have indicated that most prokaryotic genomes (>90%) consist of a single circular DNA molecule containing from 580 kbp to more than 5220 kbp of

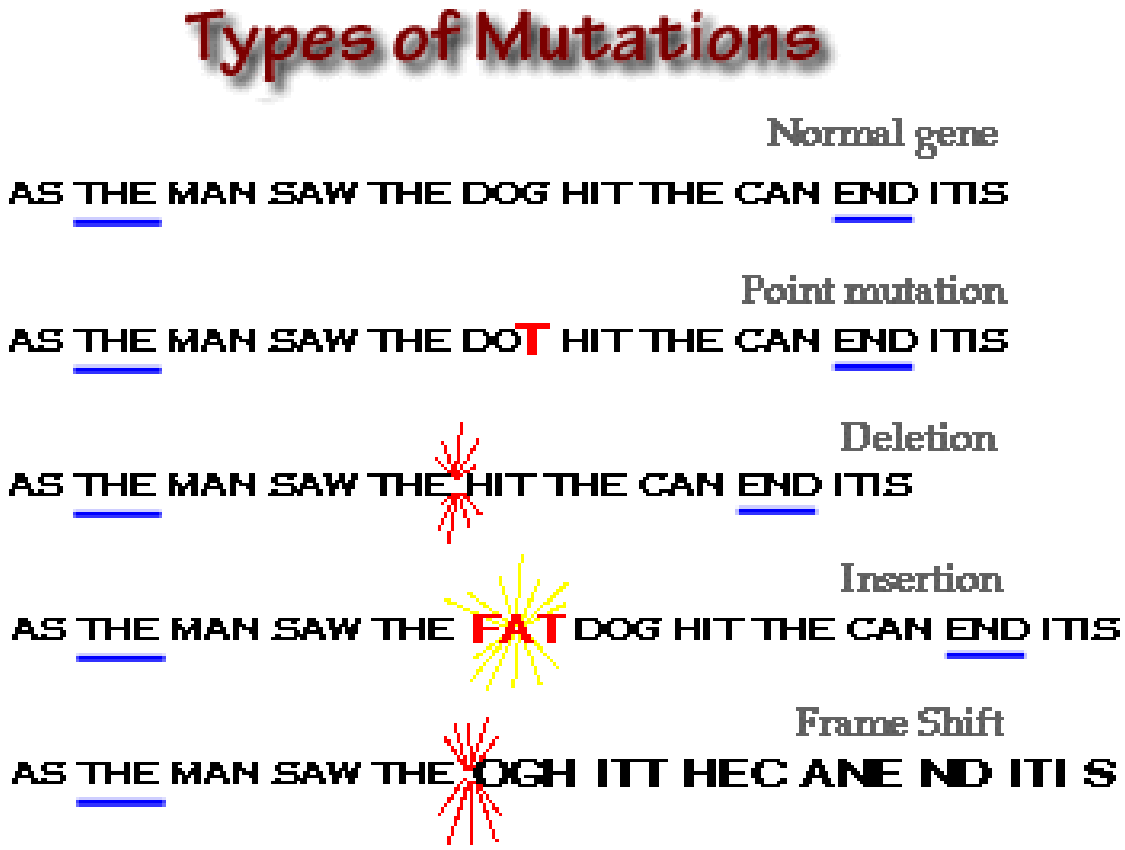
DNA. A few bacteria (eg, *Brucella melitensis*, *Burkholderia pseudomallei*, and *Vibrio cholerae*) have genomes consisting of two circular DNA molecules. Many bacteria contain additional genes on plasmids that range in size from several to 100 kbp.

EXCHANGE OF GENETIC INFORMATION

INTRODUCTION

In bacterial populations mutations are constantly arising due to errors made during replication. If there is any selective advantage for a particular mutation (e.g. antibiotic resistance), the mutant will quickly become the major component of the population due to the rapid growth rate of bacteria. In addition, since bacteria are haploid organisms, even mutations that might normally be recessive will be expressed.

Thus, mutations in bacterial populations can pose a problem in the treatment of bacterial infections. Not only are mutations a problem, bacteria having mechanisms by which genes can be transferred to other bacteria. Thus, a mutation arising in one cell can be passed on to other cells.



Bacterial genes are usually transferred to members of the same species but occasionally transfer to other species can also occur.

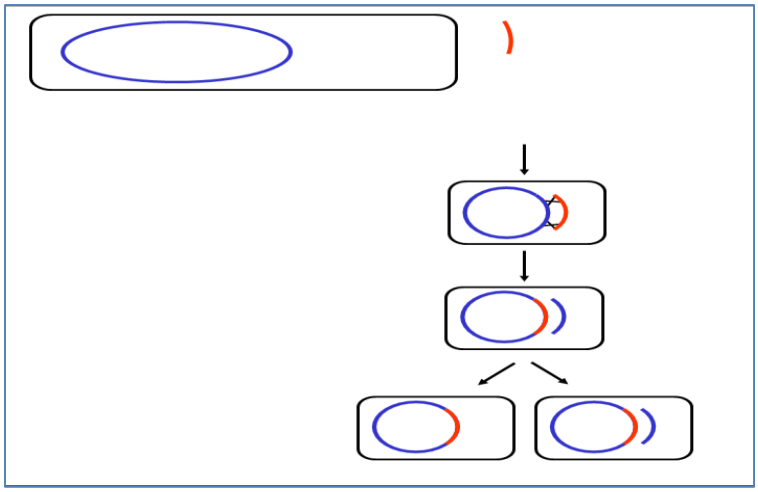
I. Restriction & Other Constraints on Gene Transfer

Restriction enzymes (restriction endonucleases) provide bacteria with a mechanism to distinguish between their own DNA and DNA from other biologic sources. These enzymes hydrolyze (cleave) DNA at restriction sites determined by specific DNA sequences ranging from four to 13 bases. DNA fragments can be selectively prepared because of this selectivity; this is the foundation of genetic engineering. Each bacterial strain that possesses a restriction system is able also to disguise these recognition sites in its own DNA by modifying them through methylation of adenine or cytosine residues within the site.

II. GENE TRANSFER MECHANISMS IN BACTERIA

A. Transformation

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. Bacillus, Haemophilus, Neisseria, and Pneumococcus) can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome.



B. Transduction

Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage. The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment.

Not all phages can mediate transduction. In most cases gene transfer is between members of the same bacterial species. However, if a particular phage has a wide

host range then transfer between species can occur. The ability of a phage to mediated transduction is related to the life cycle of the phage.

Types of Transduction according to transduced genes

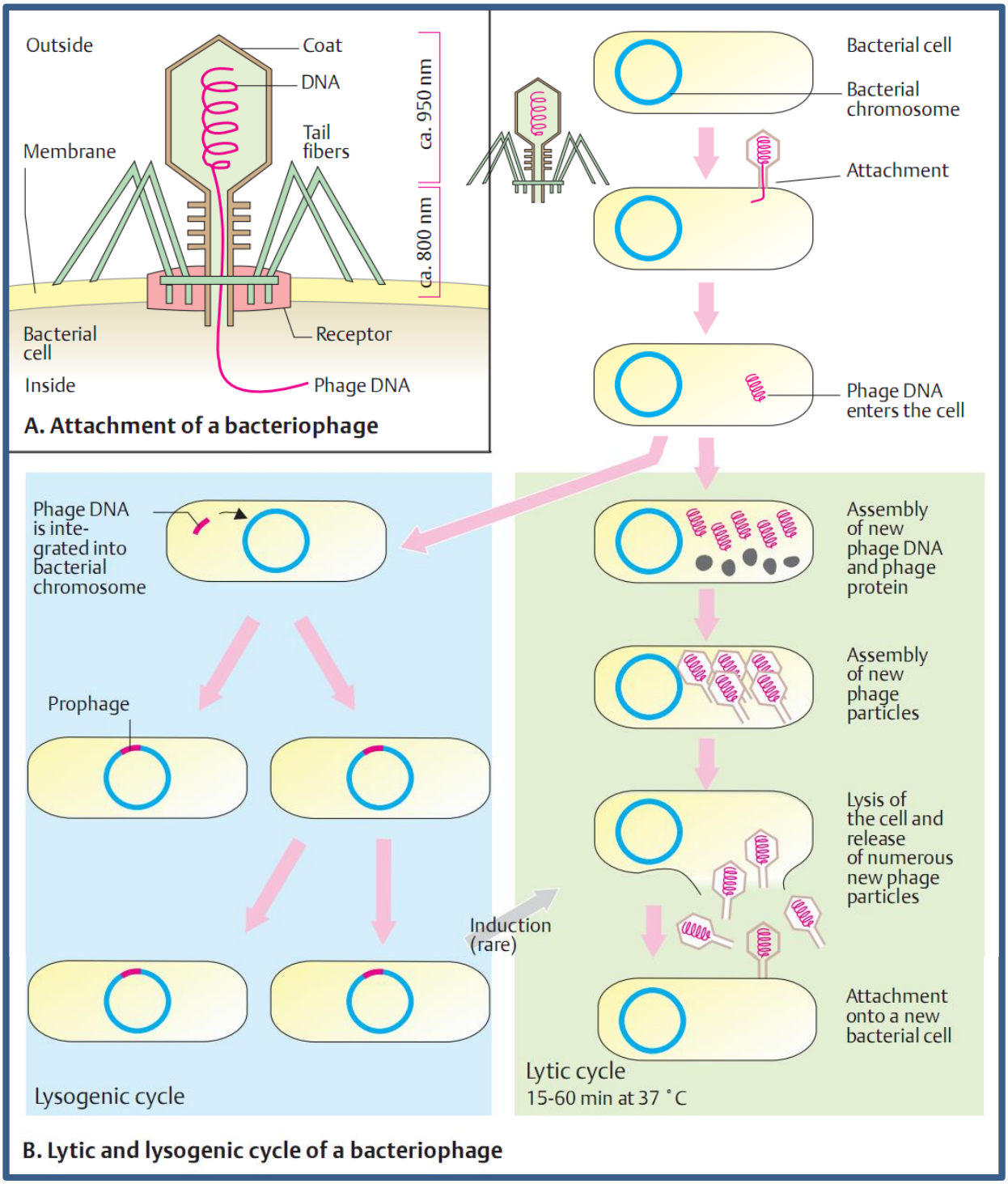


Figure: bacteriophage, structure and life cycle.

a. Generalized Transduction - Generalized transduction is transduction in which potentially **any bacterial gene** from the donor can be transferred to the recipient.
Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces and package their DNA into the phage particle by a "head-full" mechanism. Occasionally one of the pieces of host DNA is randomly packaged into a phage coat. Thus, any donor gene can be potentially transferred but only enough DNA as can fit into a phage head can be transferred. If a recipient cell is infected by a phage that contains donor DNA, donor DNA enters the recipient. In the recipient a generalized recombination event can occur which substitutes the donor DNA and recipient DNA.

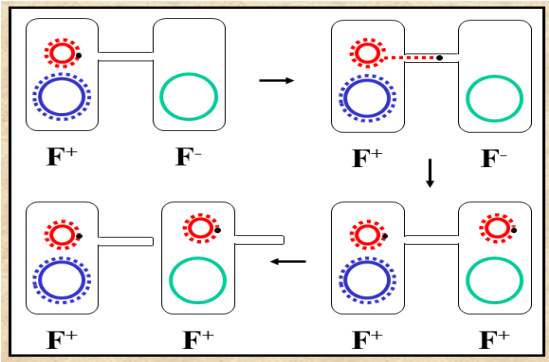
b. Specialized transduction - Specialized transduction is transduction in which **only certain donor genes** can be transferred to the recipient.
Different phages may transfer different genes but an individual phage can only transfer certain genes. Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred will depend on where the prophage has inserted in the chromosome.

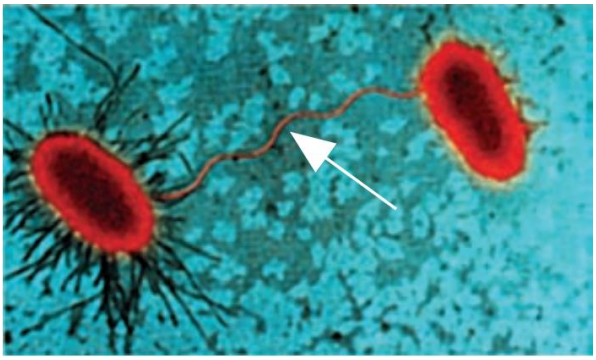
Significance

Lysogenic (phage) conversion occurs in nature and is the source of virulent strains of bacteria.

C. Conjugation

Transfer of DNA from a donor to a recipient by direct physical contact between the cells. In bacteria there are two mating types a donor (male) and a recipient (female) and the direction of transfer of genetic material is one way; DNA is transferred from a donor to a recipient .





Conjugation in bacteria

Mating types in bacteria

a. Donor

The ability of a bacterium to be a donor is a consequence of the presence in the cell of an extra piece of DNA called the F factor or fertility factor or sex factor. The F factor is a circular piece of DNA that can replicate autonomously in the cell; it is an independent replicon. Extrachromosomal pieces of DNA that can replicate autonomously are given the general name of plasmids.

The F factor has genes on it that are needed for its replication and for its ability to transfer DNA to a recipient. One of the things the F factor codes for is the ability to produce a sex pilus (F pilus) on the surface of the bacterium. This pilus is important in the conjugation process. The F factor is not the only plasmid that can mediated conjugation but it is generally used as the model.

b. Recipient

The ability to act as a recipient is a consequence of the lack of the F factor.

Significance

Among the Gram negative bacteria this is the major way that bacterial genes are transferred. Transfer can occur between different species of bacteria. Transfer of multiple antibiotics resistance by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid it is easy to see why an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one.

III. TRANSPOSABLE GENETIC ELEMENTS

Transposable genetic elements are segments of DNA that have the capacity to move from one location to another (i.e. jumping genes).

Properties of Transposable Genetic Elements:

1. Random movement
2. Not capable of self-replication
3. Transposition mediated by site-specific recombination
4. Transposition can be accompanied by duplication

Types of Transposable Genetic Elements

1. Insertion sequences (**IS**).

Insertion sequences are transposable genetic elements that carry no known genes except those that are required for transposition.

2. Transposons (**Tn**)

Transposons are transposable genetic elements that carry one or more other genes in addition to those which are essential for transposition.

Recombinant DNA technology (Genetic Engineering)

Recombinant DNA (rDNA) molecules are DNA sequences that result from the use of laboratory methods to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms.

Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure; they differ only in the **sequence of nucleotides** within that identical overall structure. Consequently, when DNA from a foreign source is linked to host sequences that can drive DNA replication and then introduced into a host organism, the foreign DNA is replicated along with the host DNA.

Recombinant DNA molecules are sometimes called chimeric DNA, because they are usually made of material from two different species, like the mythological chimera.

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, any DNA sequence may be created and introduced into any of a very wide range of living organisms.

Proteins that result from the expression of recombinant DNA within living cells are termed recombinant proteins.

Creating recombinant DNA

Construction of recombinant DNA in which, a foreign DNA fragment is inserted into a plasmid vector. In this example, the gene indicated by the white color is inactivated upon insertion of the foreign DNA fragment.

In standard cloning protocols, the cloning of any DNA fragment essentially involves seven steps: (1) Choice of host organism and cloning vector, (2) Preparation of vector DNA, (3) Preparation of DNA to be cloned, (4) Creation of recombinant DNA, (5) Introduction of recombinant DNA into the host organism, (6) Selection of organisms

containing recombinant DNA, (7) Screening for clones with desired DNA inserts and biological properties.

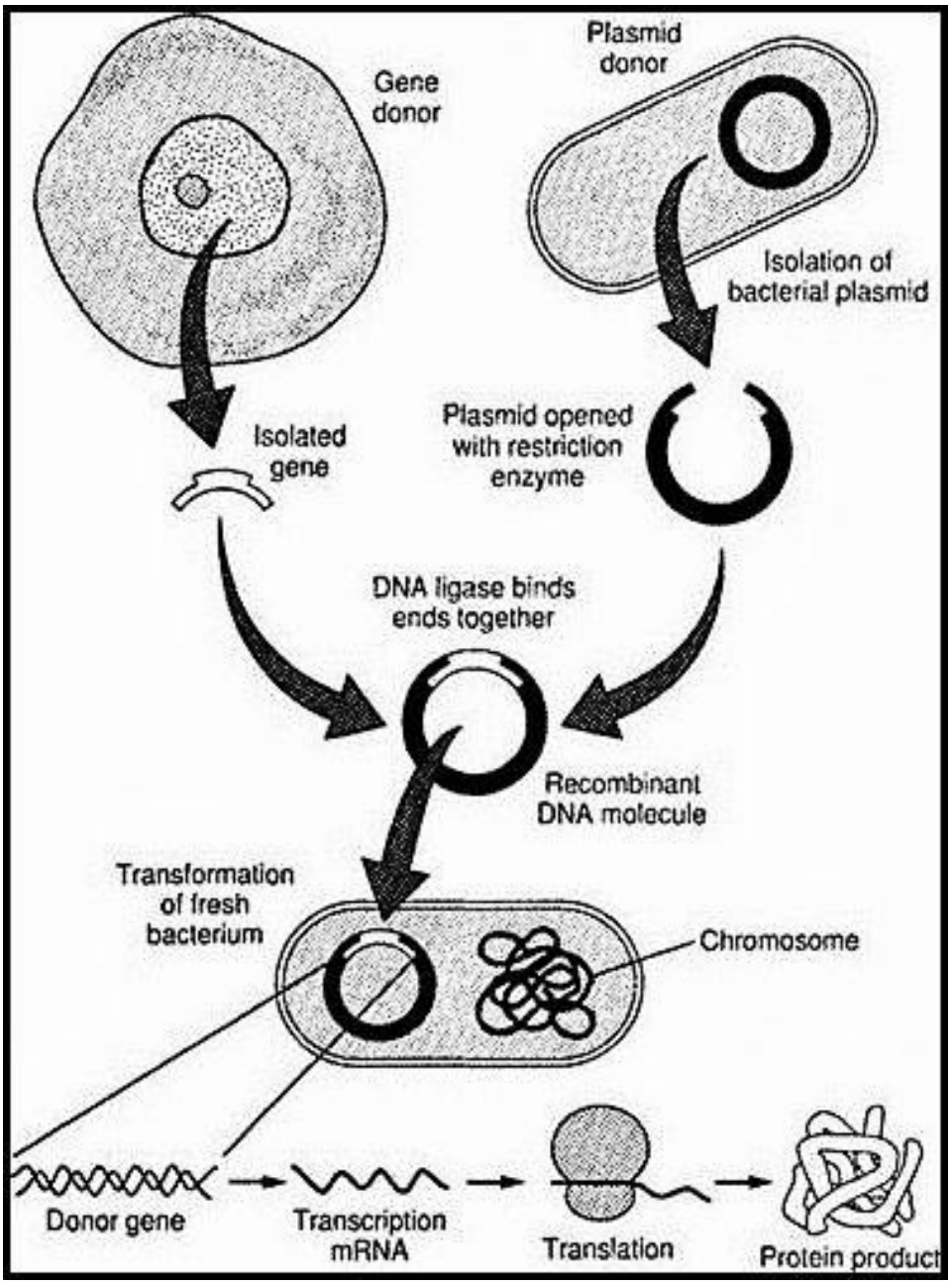


Figure : using human DNA bacteria can produce human proteins.

Applications of recombinant DNA technology

Recombinant DNA is widely used in biotechnology, medicine and research. Today, recombinant proteins and other products that result from the use of rDNA technology are found in essentially every western pharmacy, doctor's or veterinarian's office, medical testing laboratory, and biological research laboratory. In addition, organisms that have been manipulated using recombinant DNA technology, and products

derived from those organisms have found their way into many farms, supermarkets, home medicine cabinets and even pet shops.

Recombinant human insulin: Recombinant insulin has almost completely replaced insulin obtained from animal sources (e.g. pigs and cattle) for the treatment of insulin-dependent diabetes. A variety of different recombinant insulin preparations are in widespread use (DrugBank entry).

Recombinant human growth hormone (HGH, somatotropin): Growth hormone is administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development. Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers.

Recombinant blood clotting factor VIII: Recombinant factor VIII is a blood-clotting protein that is administered to patients with forms of the bleeding disorder hemophilia, who are unable to produce factor VIII in quantities sufficient to support normal blood coagulation.

Recombinant hepatitis B vaccine: Prevention of hepatitis B infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells.