# GENETIKA MIKROBA

## Struktur Gen dan Replikasi

### Chapter Overview

This chapter presents the basic concepts of molecular genetics: storage and organization of genetic information in the DNA molecule, mutagenesis, and repair. The role of microorganisms in screening procedures for mutagenic agents is also described. Primary emphasis is given to the genetics of bacteria.

### Chapter Objectives

### After reading this chapter you should be able to:

1. discuss the flow of genetic information from DNA to RNA to protein, and discuss the relationship between the nucleotide sequences of DNA and RNA and the amino acid sequences of proteins
2. discuss the nature of the genetic code
3. define a gene and discuss controlling elements, such as promoters and operators
4. discuss the four parts (promoter, leader, coding region, trailer) of a bacterial gene

**These are the most important concepts you are learning in this chapter:**

1. A gene is a nucleotide sequence that codes for a polypeptide, tRNA, or rRNA. Controlling elements such as promoters and operators often are considered part of the gene.
2. Most bacterial genes have at least four major parts, each with different functions: promoters, leaders, coding regions, and trailers.
3. DNA replication is a very complex process involving a variety of proteins and a number of steps. It is designed to operate rapidly while minimizing errors and correcting those that arise when the DNA sequence is copied.
4. Genetic information is contained in the nucleotide sequence of DNA (and sometimes RNA). When a structural gene directs the synthesis of a polypeptide, each amino acid is specified by a triplet codon.

**Study Outline**

**I. DNA as the Genetic Material**
- A. Griffith (1928) demonstrated the phenomenon of transformation: nonvirulent bacteria could become virulent when live, nonvirulent bacteria were mixed with dead, virulent bacteria
- B. Avery, MacLeod, and McCarty (1944) demonstrated that the transforming principle (the material responsible for transformation to virulence in Griffithís experiments) was DNA
- C. Hershey and Chase (1952) showed that for the T2 bacteriophage, only the DNA was needed for infectivity; therefore, they proved that DNA was the genetic material
- D. Over the past decades the relationship between DNA, RNA, and protein has been established
  1. DNA is the genetic material of cells; DNA is precisely copied by a process called replication
  2. A gene is a DNA segment that encodes a polypeptide, an rRNA, or a tRNA
  3. Genes are expressed when the information they encode is transcribed, forming an RNA molecule complementary to the original DNA template
  4. mRNA molecules direct the synthesis of proteins; the decoding of the mRNA information occurs during a process called translation

**II. DNA Replication**
- A. Pattern of DNA synthesis
  1. DNA replication is semiconservative: each strand of DNA is conserved, but the two strands are separated from each other and serve as templates for the production of another strand (according to the base-pairing rules discussed earlier)
  2. Replication forks are the areas of the DNA molecule where this strand separation occurs and the synthesis of new DNA takes place
  3. A replicon consists of an origin of replication and the DNA that is replicated as a unit from that origin
  4. The bacterial chromosome is usually a single replicon
  5. Small closed circular DNA molecules, such as plasmids and some virus genomes, replicate by means of a rolling-circle mechanism

6. The large linear DNA molecules of eucaryotes employ multiple replicons to efficiently replicate the relatively large molecules within a reasonable time span

B. Mechanism of DNA replication-as observed in E. coli
   1. DnaA protein binds to the origin of replication
   2. Helicases unwind the two strands of DNA and as they do so topoisomerases (e.g., DNA gyrase) relieve the tension caused by the unwinding process
   3. Single-stranded DNA binding proteins (SSBs) keep the single strands apart
   4. Primases synthesize a small RNA molecule (approximately 10 nucleotides) that will act as a primer for DNA synthesis
   5. DNA polymerase III synthesizes the complementary strand of DNA according to the base-pairing rules; on one strand (the leading strand), synthesis is continuous, while on the other (the lagging strand), a series of fragments are generated by discontinuous synthesis; a multiprotein complex called a replisome organizes all of these processes
   6. DNA polymerase I removes the primers and fills the gaps that result from the RNA deletion
   7. DNA ligases join the DNA fragments to form a complete strand of DNA
   8. DNA replication is extraordinarily complex; at least 30 proteins are required to replicate the E. coli chromosome
   9. The rate of DNA synthesis is 750 to 1,000 base pairs per second in procaryotes, and 50 to 100 base pairs per second in eucaryotes

**III. The Genetic Code**

A. For polypeptide-coding genes, the DNA base sequence corresponds to the amino acid sequence of the polypeptide (colinearity)

B. Establishment of the genetic code-each codon that specifies a particular amino acid must be three bases long for each of the 20 amino acids to have at least one codon; thus the genetic code consists of 64 codons

C. Organization of the code
   1. Degeneracy-many amino acids are encoded by more than one codon
   2. Sense codons-61 codons that specify amino acids
   3. Stop (nonsense) codons-three codons (UGA, UAG, UAA) that do not specify an amino acid, and that are used as translation (protein synthesis) termination signals
   4. Wobble-describes the somewhat loose base pairing of a tRNA anticodon to the mRNA codon; wobble eliminates the need for a unique tRNA for each codon because the first two positions are sufficient to establish hydrogen bonding between the mRNA and the aminoacyl-tRNAs

**IV. Gene Structure**

A. Gene-a linear sequence of nucleotides that is within the genomic nucleic acid molecule, and that has a fixed start point and end point
   1. Encodes a polypeptide, a tRNA, or an rRNA
   2. Has controlling elements (e.g., promoters) that regulate expression of a gene; may be considered as part of the gene itself, or they may be considered as separate regulatory sequences
   3. With some exceptions, genes are not overlapping
   4. The segment that encodes a single polypeptide is also called a cistron
   5. In procaryotes-coding information is normally continuous although some bacterial genes are interrupted; in eucaryotes-most genes have coding sequences (exons) that are interrupted by noncoding sequences (introns)

B. Genes that code for proteins
   1. Template strand-the one strand that contains coding information and directs RNA synthesis
   2. Promoter-a sequence of bases that is usually situated upstream from the coding region; serves as a recognition/binding site for RNA polymerase
      a. Recognition site-site of initial association with RNA polymerase (35 bases upstream of transcription initiation site)
      b. Binding site (Pribnow box)-sequence that favors DNA unwinding before transcription begins (approximately 10 bases upstream of transcription initiation site)
      c. Consensus sequences-idealized base sequences found most often when comparing the sequences of different bacteria
   3. Leader sequence-a transcribed sequence that is not translated; contains a consensus sequence known as the Shine-Dalgarno sequence, which serves as the recognition site for the ribosome
   4. Coding region-the sequence that begins immediately downstream of the leader sequence; starts with the template sequence 3¢TAC5¢, which gives rise to mRNA codon 5¢AUG3¢, the first translated codon (specifies N-formylmethionine in bacteria, methionine in archaea and eucaryotes)
   5. Trailer region-nontranslated region located immediately downstream of the translation terminator sequence and before the transcription terminator

6. Regulatory sites-sites where DNA-recognizing regulatory proteins bind to either stimulate or inhibit gene expression

C. Genes that code for tRNA and rRNA
   1. tRNA genes-promoters, leaders, coding regions, and trailer regions are found; noncoding regions are removed after transcription; more than one tRNA may be made from a single transcript; the tRNAs are separated by a noncoding spacer region, which is removed after transcription
   2. rRNA genes-have promoters, leaders, coding regions and trailer regions; all rRNA molecules are transcribed as a single large transcript, which is cut up after transcription, yielding the final rRNA products

# Genes: Expression and Regulation

**Chapter Overview**

This chapter presents an overview of the synthesis of RNA and proteins, the two processes involved in gene expression. The last part of the chapter considers a variety of mechanisms by which gene expression is regulated. The discussion focuses on the control of transcription as most regulation of gene expression occurs at this level.

**Chapter Objectives**

After reading this chapter you should be able to:

1. discuss the synthesis of RNA
2. describe the similarities and differences between eucaryotic and procaryotic RNA transcription
3. discuss the synthesis of proteins and describe the role(s) of the various components required for this process
4. describe induction and repression
5. discuss regulation of enzyme synthesis by negative and positive regulatory proteins
6. describe attenuation and contrast this regulatory mechanism with repression and derepression of the synthesis of biosynthetic enzymes
7. discuss global regulation of gene expression
8. describe two-component phosphorelay systems
9. discuss regulation of the cell cycle

**These are the most important concepts you are learning in this chapter:**

1. In transcription the RNA polymerase copies the appropriate sequence on the DNA template sense strand to produce a complementary RNA copy of the gene. Transcription differs in a number of ways between procaryotes and eukaryotes, even though the basic mechanism of RNA polymerase action is essentially the same.
2. The flow of genetic information usually proceeds from DNA through RNA to protein. A protein's amino acid sequence reflects the nucleotide sequence of its mRNA. This messenger is a complementary copy of a portion of the DNA genome. Metabolism is regulated in such a way that (a) cell components are maintained at the proper concentrations, even in the face of a changing environment, and (b) energy and material are conserved.
3. The localization of enzymes and metabolites in separate compartments of a cell regulates and coordinates metabolic activity.
4. The activity of regulatory enzymes may be changed through reversible binding of effectors to a regulatory site separate from the catalytic site or through covalent modification of the enzyme. Regulation of enzyme activity operates rapidly and serves as a fine-tuning mechanism to adjust metabolism from moment to moment.
5. A pathway's activity is often controlled by its end products through feedback inhibition of regulatory enzymes located at the start of the sequence and at branch points.
6. The long-term regulation of metabolism in bacteria is achieved through the control of transcription by such mechanisms as sigma factors, repressor proteins during induction and repression, and by the attenuation of many biosynthetic operons.

## Study Outline

**I. DNA Transcription or RNA Synthesis**

A. Transcription-the synthesis of RNA under the direction of DNA
   1. The RNA product is complementary to the DNA template
   2. An adenine nucleotide in the DNA template directs the incorporation of a uracil nucleotide in the RNA; otherwise, the base pair rules are the same as for DNA replication
   3. Three types of RNA are produced by transcription
      a. mRNA carries the message that directs the synthesis of proteins
      b. tRNA carries amino acids during protein synthesis
      c. rRNA molecules are components of the ribosomes

B. Transcription in procaryotes
   1. Procaryotic mRNA can code for one polypeptide (monogenic) or many polypeptides (polygenic); in addition to coding regions, mRNA molecules may have untranslated regions
      a. Leader sequences consist of 25 to 150 bases at the 5¢ end of the mRNA, and precede the initiation codon
      b. Spacer regions separate the segments that code for individual polypeptides in polygenic mRNAs
      c. Trailer regions are found at the 3¢ end of the mRNA after the last termination codon
   2. RNA polymerase (a large multi-subunit enzyme) is responsible for the synthesis of RNA
      a. The core enzyme (a2,b,b' subunits) catalyzes RNA synthesis
      b. The sigma subunit (sigma factor) is not catalytic, but helps the core enzyme bind DNA at the appropriate site
   3. A promoter is the region of the DNA to which RNA polymerase binds in order to initiate transcription; sequences centered at 35 and 10 base pairs before the transcription starting point are important in directing RNA polymerase to the promoter
   4. Terminators are regions of the DNA that signal termination of the transcription process

C. Transcription in eucaryotes
   1. There are three major RNA polymerases
      a. RNA polymerase II-catalyzes mRNA synthesis; it requires several initiation factors and recognizes promoters that have several important elements (rather than just two as seen in procaryotes)
      b. RNA polymerase I-catalyzes rRNA (5.8S, 18S, and 28S) synthesis
      c. RNA polymerase III-catalyzes tRNA and 5S rRNA synthesis
   2. Transcription yields large monogenic RNA precursors (heterogeneous nuclear RNA; hnRNA) that must be processed by posttranscriptional modification to produce mRNA
      a. Adenylic acid is added to the 3' end to produce a polyA sequence about 200 nucleotides long (polyA tail)
      b. 7-methylguanosine is added to the 5' end by a tri-phosphate linkage (5' cap)
      c. These two modifications are believed to protect the mRNA from exonuclease digestion
   3. Eucaryotic genes are split or interrupted such that the expressed sequences (exons) are separated from one another by intervening sequences (introns); the introns are represented in the primary transcript but are subsequently removed by a process called RNA splicing; some splicing is self-catalyzed by RNA molecules called ribozymes; interrupted genes have been found in cyanobacteria and archaea, but not in other procaryotes

**II. Protein Synthesis**

A. Translation-the synthesis of a polypeptide chain directed by the nucleotide sequence in a mRNA molecule
   1. Ribosome-site of translation
   2. Polyribosome-complex of mRNA with several ribosomes

B. Transfer RNA and amino acid activation
   1. The first stage of protein synthesis is the attachment of amino acids to tRNA molecules (catalyzed by aminoacyl-tRNA synthetase); this process is referred to as amino acid activation
   2. Each tRNA has an acceptor end and can only carry a specific amino acid; it also has an anticodon triplet that is complementary to the mRNA codon triplet

C. The ribosome-complex organelle constructed from several rRNA molecules and many polypeptides; has two subunits (in procaryotes: 50S and 30S)
D. Initiation of protein synthesis
    1. fMet-tRNA (in bacteria, Met-tRNA in archaea and eucaryotes) binds the small subunit of the ribosome; they bind the mRNA at a special initiator codon (AUG); then the large subunit of the ribosome binds
    2. Three protein initiation factors are also required in procaryotes (eucaryotes require more initiation factors)
E. Elongation of the polypeptide chain
    1. Elongation involves the sequential addition of amino acids to the growing polypeptide chain; several polypeptide elongation factors are required for this process
    2. The ribosome has three sites for binding tRNA molecules: peptidyl site (P site), aminoacyl site (A site), and exit site (E site)
    3. Each new amino acid is positioned in the A site by its tRNA, which has an anticodon that is complementary to the codon on the mRNA molecule
    4. The ribosomal enzyme peptidyl transferase catalyzes the formation of the peptide bonds between adjacent amino acids; the 23S rRNA is a major component of this enzyme
    5. After each amino acid is added to the chain, translocation occurs and thereby moves the ribosome to position the next codon in the A site
F. Termination of protein synthesis
    1. Takes place at any one of three special codons (UAA, UAG, or UGA)
    2. Three polypeptide release factors aid in the recognition of these codons
    3. The ribosome hydrolyzes the bond between the completed protein and the final tRNA, and the protein is released from the ribosome, which then dissociates into its two component subunits
G. Protein synthesis is expensive, using five high-energy bonds to add one amino acid to the chain
H. Protein folding and molecular chaperones
    1. Molecular chaperones-special helper proteins that aid the nascent polypeptide in folding to its proper shape; many have been identified and they include heat shock proteins and stress proteins; in addition to helping polypeptides fold, chaperones are important in the transport or protein across membranes
    2. Protein conformation is a direct function of amino acid sequence; proteins have self-folding, structurally independent regions called domains
I. Protein splicing-before folding, part of the polypeptide is removed; such splicing removes intervening sequences (inteins) from the sequences (exteins) that remain in the final product

**III. Regulation of mRNA Synthesis**

A. Regulation of mRNA synthesis (and thereby enzyme synthesis) provides a long-term regulatory mechanism that very effectively conserves energy and raw material and maintains overall balance of cell proteins in response to major changes in environmental conditions; it complements but is less rapid than regulation of enzyme activity
B. Induction and repression
    1. Synthesis of enzymes involved in catabolic pathways can be inducible, and the initial substrate of the pathway (or some derivative of it) is usually the inducer; induction increases the amount of mRNA encoding the enzymes
    2. Synthesis of enzymes involved in anabolic pathways is repressible and the end product of the pathway usually acts as a corepressor; repression decreases the amount of mRNA encoding the enzymes
C. Negative control
    1. The rate of mRNA synthesis is controlled by repressor proteins, which bind to specific sites on the DNA called operators
    2. In inducible systems, the repressor protein is active until bound to the inducer (binding of inducer inactivates the repressor) whereas in repressible systems, the repressor is inactive until bound to the corepressor (binding of corepressor activates the repressor)
    3. In bacteria, a set of structural genes controlled by a particular operator and promoter is called an operon
    4. The lactose operon is an excellent example of negative regulation; binding of the lac repressor to the lac operator may either inhibit RNA polymerase binding or block the movement of RNA polymerase
D. Positive control
    1. Positive control occurs when an operon can function only in the presence of a controlling factor

2. The lactose operon is regulated by catabolite activator protein (CAP); the activity of CAP is regulated by cAMP; cAMP activates CAP so that it binds a specific site on the DNA, stimulating transcription

**IV. Attentuation**

A. There are two decision points for regulating transcription of anabolic pathways: initiation and continuation of transcription; attenuation regulates continuation of transcription

B. In systems where transcription and translation are tightly coupled, ribosome behavior in the leader region of the mRNA can control continuation of transcription
   1. If ribosomes actively translate the leader region (attenuator), which contains several codons for the amino acid product of the operon, a transcription terminator forms and transcription will not continue
   2. If ribosomes stall during translation of the leader region because the appropriate charged aminoacyl-tRNA is absent, the terminator does not form and transcription will continue

**V. Global Regulatory Systems**

A. Overview
   1. Global regulatory systems-systems that affect many genes and pathways simultaneously, allowing for both independent regulation of operons as well as cooperation of operons
   2. Global regulation can be accomplished by several mechanisms, including the use of a single regulator protein (repressor or activator) to regulate several operons, use of different sigma factors, and the use of nonprotein regulators
   3. Regulon-a collection of genes or operons controlled by a common regulatory protein

B. Catabolite repression
   1. Diauxic growth-a biphasic growth pattern observed when a bacterium is grown on two different sugars (e.g., glucose and lactose)
   2. For E. coli, availability of glucose (the preferred carbon and energy source) causes a drop in cAMP levels, resulting in the deactivation of CAP (a positive regulator of several catabolic pathways, including the lactose operon); deactivation of CAP allows the bacterium to use glucose preferentially over another sugar when both are present in the environment

C. Regulation by sigma factors and control of sporulation
   1. Bacteria produce a number of different sigma factors; each enables RNA polymerase to recognize and bind to specific promoters
   2. Alteration of the sigma factors available to RNA polymerase changes gene expression
   3. This has been demonstrated in a number of systems including heat shock response in E. coli and sporulation in B. subtilis

D. Regulation by antisense RNA and the control of porin proteins-antisense RNA is complementary to an RNA molecule and specifically binds to it, thereby blocking DNA replication, mRNA synthesis, or translation, depending on the nature of the RNA target

**VI. Two-Component Phosphorelay Systems**

A. A signal transduction system that uses transfer of phosphoryl groups to control gene transcription and protein activity

B. In the sporulation regulation system, sequential transfer of a phosphoryl group from a sensor kinase to a transcription regulator, via two other proteins, allows the bacterium to respond to environmental conditions

C. Chemotaxis-in this system, attractants (or repellants) are detected by chemotactic proteins, leading to transfer of phsophoryl groups to proteins that regulate the direction of rotation of the bacterial flagellum

**VII. Control of the Cell Cycle**

A. The complete sequence of events extending from the formation of a new cell through the next division is called the cell cycle: it requires tight coordination of DNA replication and cell division

B. There appear to be two separate controls for the cell cycle, one sensitive to cell mass and the other responding to cell length

C. In E. coli, regulation of DNA replication involves DnaA protein, which binds to the origin of replication to initiate replication

D. The initiation of septation requires both termination of DNA replication and the attainment of threshold lengths; how this occurs in unknown

E. In rapidly growing bacterial cultures, initiation of a round of DNA replication can begin before a round of replication is finished

# Microbial Recombination and Plasmids

**Chapter Overview**

This chapter begins with a general discussion of bacterial recombination, plasmids, and transposable elements, and then examines the acquisition of genetic information by conjugation, transformation, and transduction. The way these recombination procedures are used to map the bacterial genome is explained. Finally, viral recombination and genome mapping are discussed.

**Chapter Objectives**

After reading this chapter you should be able to:

1. discuss the nature of procaryotic recombination
2. distinguish horizontal gene transfer from vertical gene transfer
3. compare and contrast conjugation, transformation, and transduction
4. discuss how transposable elements can move genetic material between bacterial chromosomes and within a chromosome to cause changes in the genome and the phenotype of the organism
5. discuss the use of conjugation, transformation, and transduction to map the bacterial genome
6. discuss the methods used to map viral genes

**These are the most important concepts you are learning in this chapter:**

1. Recombination is a one-way process in procaryotes: a piece of genetic material (the exogenote) is donated to the chromosome of a recipient cell (the endogenote) and integrated into it.
2. The actual transfer of genetic material between bacteria usually takes place in one of three ways: direct transfer between two bacteria temporarily in physical contact (conjugation), transfer of a naked DNA fragment (transformation), or transport of bacterial DNA by bacteriophages (transduction).
3. Plasmids and transposable elements can move genetic material between bacterial chromosomes and within chromosomes to cause rapid changes in genomes and drastically alter phenotypes.
4. The bacterial chromosome can be mapped with great precision, using Hfr conjugation in combination with transformational and transductional mapping techniques.
5. The DNA sequences of complete genomes are now being determined. Analysis of these sequences is telling us much about microbial physiology and phylogeny.
6. Recombination of virus genomes occurs when two viruses with homologous chromosomes infect a host cell at the same time

**Study Outline**

**I. Introduction**
- A. Recombination-process by which one or more nucleic acid molecules are rearranged or combined to produce a new nucleotide sequence
- B. In eucaryotes, recombination occurs during meiosis and results from crossing-over between homologous chromosomes (chromosomes containing identical sequences of genes)

**II. Bacterial Recombination: General Principles**
- A. Types of recombination
  1. General recombination usually involves a reciprocal exchange in which a pair of homologous sequences breaks and rejoins in a crossover; nonreciprocal general recombination involves the incorporation of a single strand into the chromosome to form a stretch of heteroduplex DNA
  2. Site-specific recombination is the nonhomologous insertion of DNA into a chromosome; often occurs during viral genome integration into the host, a process catalyzed by enzymes specific for the virus and its host
  3. Replicative recombination accompanies replication and is used by some genetic elements that move about the genome
- B. Horizontal gene transfer-transfer of genes from one mature, independent organism to another (compare this to vertical gene transfer-transmission of genes from parents to offspring)
  1. Exogenote-donor DNA that enters the bacterium by one of several mechanisms
  2. Endogenote-the genome of the recipient

3. Merozygote-a recipient cell that is temporarily diploid for a portion of the genome during the gene transfer process
4. Most linear DNA fragments are not stably maintained unless integrated into the endogenote

C. Types of horizontal exogenote transfer
1. Conjugation is direct transfer from donor bacterium to recipient while the two are temporarily in physical contact
2. Transformation is transfer of a naked DNA molecule
3. Transduction is transfer mediated by a bacteriophage

D. Intracellular fates of exogenote
1. Integration into the host chromosome
2. Independent functioning and replication of the exogenote without integration (a partial diploid clone develops)
3. Survival without replication (only the one cell is a partial diploid)
4. Degradation by host nucleases (host restriction)

**III. Bacterial Plasmids**

A. Plasmids-small, circular DNA molecules that are not part of the bacteriumís chromosome
1. Have their own replication origins, replicate autonomously, and are stably inherited
2. Can be eliminated from a cell by a process called curing, which can occur either spontaneously or as a result of treatments that inhibit plasmid replication but do not affect host cell reproduction
3. Episomes are plasmids that can exist either with or without being integrated into the host chromosome
4. Conjugative plasmids usually have genes for sex pili and can transfer copies of themselves to other bacteria during conjugation

B. Fertility factors-episomes that can direct the formation of sex pili and transfer copies of themselves during conjugation

C. Resistance factors-(R plasmids); have genes for resistance to various antibiotics; some are conjugative; however, they usually do not integrate into the host chromosome

D. Col plasmids-provide a competitive advantage to the bacteria having them; carry genes for the synthesis of bacteriocins (e.g., colicins) that are directed against other bacterial species; some col plasmids are conjugative and may also carry resistance genes

E. Other types of plasmids include virulence plasmids, which make the bacterium more pathogenic by conferring resistance to host defense mechanisms or by carrying a gene for the production of a toxin, and metabolic plasmids, which carry genes for enzymes that utilize certain substances as nutrients (aromatic compounds, pesticides, etc.)

**IV. Transposable Elements**

A. Transposition-the movement of pieces of DNA around in the genome
1. Transposons-segments of DNA that can move about chromosomes
2. Transposons differ from bacteriophages in that they lack an infectious viral life cycle, and they differ from plasmids in that they are unable to replicate independently

B. Insertion sequences (IS elements) contain genes only for those enzymes required for transposition (e.g., transposase); they are bound on both ends by inverted terminal repeat sequences

C. Composite transposons carry other genes in addition to those needed for transposition (e.g., for antibiotic resistance, toxin production, etc.)

D. Movement of the transposon occurs typically by replicative transposition, during which a replicated copy of the transposon inserts at the target site on the DNA, while the original copy remains at the parental site

E. Effects of transposable elements
1. Insertional mutagenesis can cause deletion of genetic material at or near the target site, arrest of translation or transcription due to stop codons or termination sequences located on the inserted material, and activation of genes near the point of insertion due to promoters located on the inserted material
2. Fusion of plasmids and insertion of F plasmids into chromsosome
3. Generation of plasmids with resistance genes
4. Conjugative transposons can move between bacteria through the process of conjugation

F. Transposable elements are present in eucaryotes, bacteria, and archaea

**V. Bacterial Conjugation**

A. The transfer of genetic information via direct cell-cell contact; this process is mediated by fertility factors (F plasmids)

B. F+ ¥ F- mating

1. In E. coli and other gram-negative bacteria, an F plasmid moves from the donor (F+) to a recipient (F-) while being replicated by the rolling circle mechanism
    a. The displaced strand is transferred via a sex pilus and then copied to produce double-stranded DNA; the donor retains the other parental DNA strand and its complement; thus the recipient becomes F+ and the donor remains F+
    b. Chromosomal genes are not transferred
2. In gram-positive bacteria, the sex pilus is not necessarily required for transmission; generally fewer genes are transferred

C. Hfr conjugation
1. F plasmid integration into the host chromosome results in an Hfr strain of bacteria
2. The mechanics of conjugation of Hfr strains are similar to those of F+ strains
3. The initial break for rolling-circle replication is at the integrated plasmidís origin of transfer site
    a. Part of the plasmid is transferred first
    b. Chromosomal genes are transferred next
    c. The rest of the plasmid is transferred last
4. Complete transfer of the chromosome takes approximately 100 minutes, but the conjugation bridge does not usually last that long; therefore, the entire F factor is not usually transferred, and the recipient remains F-

D. F¢ conjugation (sexduction)
1. When an integrated F plasmid leaves the chromosome incorrectly, it may take with it some chromosomal genes from one side of the integration site; this results in the formation of an abnormal plasmid called an F¢ plasmid
2. The F¢ cell (cell harboring an F¢ plasmid) retains its genes, although some of them are in the chromosome and some are on the plasmid; in conjugation, an F¢ cell behaves as an F+ cell, mating only with F- cells
3. The chromosomal genes included in the plasmid are transferred with the rest of the plasmid, but other chromosomal genes usually are not
4. The recipient becomes an F¢ cell, and a partially diploid merozygote

**VI. DNA Transformation**

A. Transformation-a naked DNA molecule from the environment is taken up by the cell and incorporated into its chromosome in some heritable form

B. A competent cell is one that is capable of taking up DNA and therefore acting as a recipient; only a limited number of species are naturally competent; the mechanics of the natural transformation process differ from species to species

C. Species that are not normally competent (such as E. coli) can be made competent by calcium chloride treatment or other methods, which makes the cells more permeable to DNA

**VII. Transduction**

A. Transduction-transfer of bacterial genes by viruses (bacteriophages); occurs as the result of the reproductive cycle of the virus
1. Lytic cycle-a viral reproductive cycle that ends in lysis of the host cell; viruses that use this cycle are called virulent bacteriophages
2. Lysogeny-a reproductive cycle that involves maintenance of the viral genome (prophage) within the host cell (usually integrated into the host cellís chromosome), without immediate lysis of the host; with each round of cell division, the prophage is replicated and inherited by daughter cells; bacteriophages reproducing by this mechanism are called temperate phages; certain stimuli (e.g., UV radiation) can trigger the switch form lysogeny to the lytic cycle

B. Generalized transduction-any part of the bacterial genome can be transferred; occurs during the lytic cycle of virulent and temperate bacteriophages
1. The phage degrades host chromosome into randomly sized fragments
2. During assembly, fragments of host DNA of the appropriate size can be mistakenly packaged into a phage head (generalized transducing particle)
3. When the next host is infected, the bacterial genes are injected and a merozygote is formed
    a. Preservation of the transferred genes requires their integration into the host chromosome
    b. Much of the transferred DNA does not integrate into the host chromosome, but is often able to survive and be expressed; the host is called an abortive transductant

C. Specialized (restricted) transduction

1. Transfer of only specific portions of the bacterial genome; carried out only by temperate phages that have integrated their DNA into the host chromosome at a specific site in the chromosome
    a. The integrated prophage is sometimes excised incorrectly and contains portions of the bacterial DNA that was adjacent to the phageís integration site on the chromosome
    b. The excised phage genome is defective because some of its own genes have been replaced by bacterial genes; therefore, the bacteriophage cannot reproduce
    c. When the next host is infected, the donor bacterial genes are injected, leading to the formation of a merozygote
2. Low frequency transduction lysates-lysates containing mostly normal phages and just a few specialized transducing phages
3. High frequency transduction lysates-lysates containing a relatively large number of specialized transducing phages; created by coinfecting a host cell with a helper phage (normal phage) and a transducing phage; the helper phage allows the transducing phage to replicate, thus increasing the number of transducing phages in the lysate

**VIII. Mapping the Genome**

A. Hfr mapping involves the use of an interrupted mating experiment to locate the relative position of genes
    1. The conjugative bridge is broken and the Hfr ¥ F- mating is stopped at various intervals
    2. While the bridge is intact, chromosome transfer occurs at a constant rate
    3. The order and timing of gene tranfser directly reflects the order of genes on the chromosome
    4. Interrupted mating is good for mapping genes that are 3 minutes or more apart; however the instability of the conjugation bridge makes it nearly impossible to map genes that are very distant; several Hfr strains with different integration sites are used to generate overlapping maps, which can then be pieced together to form the entire genome map
B. Transformation mapping-the frequency with which two genes simultaneously transform a recipient cell (cotransformation) indicates the distance between the genes; the higher the frequency of cotransformation, the closer the two genes are; overlapping maps can be pieced together to complete the genome map
C. Generalized transduction maps-as with transformation mapping, the frequency of cotransduction indicates the distance of two genes from each other
D. Specialized transduction maps provide distances from integration sites, which themselves must be mapped by conjugation mapping techniques

**IX. Recombination and Genome Mapping in Viruses**

A. Recombination maps are generated from crossover frequency data obtained when cells are infected with two or more phage particles simultaneously
B. Heteroduplex mapping-wild type and mutant chromosomes are denatured and allowed to reanneal together; homologous regions pair normally, but mutant regions form bubbles that can be seen in electron micrographs; generates a physical map of the viral genome
C. Restriction endonuclease mapping-locates deletions and other mutations by examining the electrophoretic mobility (size) of the fragments generated
D. Sequence mapping-small phage genomes can be directly sequenced to map genes