Chapter 8: Microbial Genetics

Introduction

- Genetics is the science of heredity.
  - Study of genes:
    - How they carry information
    - How they are replicated
    - How they are passed from one organism to another
    - How they are expressed
  - Genes: Segment of DNA (or RNA in some viruses) that codes for functional products.
  - Chromosomes: Cellular structures that carry hereditary information. They contain all or most of an individual’s genes.

Structure and Function of Genetic Material

- DNA is genetic material in all living organisms.
- RNA is genetic material of some viruses.
- DNA is a macromolecule made of repeating units called nucleotides.
- Each DNA nucleotide has a nitrogenous base (adenine, cytosine, guanine, or thymine), a sugar (deoxyribose), and a phosphate group.
- DNA strands are held together by hydrogen bonds between nitrogenous bases.
  - Cytosine pairs with Guanine (C = G)
  - Thymine pairs with Adenine (A = T)
- DNA strands are complementary.
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Gene Expression

- DNA codes for proteins and RNA products.
- The flow of genetic information is:
  - Transcription        Translation
  - DNA --> RNA --> Protein
- DNA only has 4 different nucleotides; while proteins have 20 different amino acids.
- Genetic Code: Each amino acid is determined by a group of 3 nucleotides (triplet or codon). There are a total of 64 (4^3 = 4 x 4 x 4) possible codons.
- To express genetic information, DNA structure must be disrupted.

Gene Expression (Continued)

- DNA sequence must be replicated (duplicated) each time a cell divides.
- During DNA replication, two identical daughter molecules are made.
- DNA can change or mutate during replication. Some mutations are harmless, others are harmful, and a few may be beneficial.
Genotype and Phenotype

- **Genotype**: The genetic makeup of an individual. The information that codes for that organism's genetic characteristics. Collection of an individual's genes.
- **Phenotype**: Expressed properties of an individual. An individual's phenotype is a function of the genotype (and environment). Collection of an individual's proteins or gene products.

DNA and Chromosomes

- **Bacteria**: Typically have a single circular chromosome that is attached to the plasma membrane. 
  - *E. coli* chromosome is 4 million base pairs and contains about 2000 genes.
- **Eukaryotes**: Typically have several linear chromosomes that are inside the nucleus.
  - Humans have 46 chromosomes with a total length of 3 billion base pairs, which code for up to 100,000 different genes.

DNA Replication

- One parent double stranded molecule generates two daughter strands.
- In bacteria, replication begins at an origin of replication.
- Replication is semiconservative. Each strand acts as a template for the production of a new strand. Each new DNA molecule has one old strand and one new strand.
- DNA strands are antiparallel.
  - One strand goes from 5' to 3' direction.
  - Opposite strand goes from 3' to 5' direction.
- DNA polymerase only synthesizes in 5' to 3' direction.
- Leading strand is synthesized continuously.
- Lagging strand is synthesized in small fragments, of about 1000 nucleotides.

Steps in DNA Replication

1. Enzymes unwind double stranded DNA molecule.
2. Proteins stabilize unwound DNA.
3. **Leading strand** is synthesized continuously by DNA polymerase in 5' to 3' direction.
4. **Lagging strand** is synthesized discontinuously.
5. RNA primers are made by RNA polymerase and extended by DNA polymerase. Okazaki fragments: RNA-DNA fragments.
6. DNA polymerase digests RNA primers and replaces them with DNA.
7. DNA ligase joins discontinuous fragments of lagging strand.
- **Error Rate**: 1 out of $10^{10}$ bases is changed (mutation). DNA polymerase has proofreading mechanism.

Circular Chromosome of *E. coli*

DNA Replication is Semiconservative
**DNA Replication: Leading and Lagging Strands Are Copied Differently**

- Enzymes unwind the parental double helix.
- Proteins stabilize the unwound parental DNA.
- The leading strand is synthesized continuously by DNA polymerase.
- The lagging strand is synthesized discontinuously by DNA polymerase.

**RNA Synthesis (Transcription)**

There are three types of RNA in bacterial cells:

- **mRNA**: Messenger RNA. Carries information for protein synthesis.
- **rRNA**: Ribosomal RNA. Forms part of ribosome.
- **tRNA**: Transfer RNA. Carries amino acids to growing protein during translation.

**Steps of Transcription**

1. RNA polymerase binds to DNA sequence called promoter.
2. RNA polymerase makes RNA copy of gene (transcript).
3. RNA synthesis continues until RNA polymerase reaches a terminator.
4. New RNA molecule and RNA polymerase are released.

**Process of Transcription: DNA to RNA**

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**Protein Synthesis (Translation)**

- mRNA is used to make protein.
- mRNA is read in codons or nucleotide triplets.
- Genetic Code was cracked in 1960s. There are 64 possible codons, 20 amino acids.
- **AUG**: Start codon (Methionine)
- **UAA, UGA, UAG**: Stop codons
- Translation occurs on the ribosome, which is made up of two subunits (large and small).
- tRNA molecules have an **anticodon**, which recognizes codons. They carry specific amino acids to the growing protein chain.

**Steps of Translation**

1. **Initiation**: Ribosomal subunits and mRNA assemble.
2. **Start codon (AUG)** binds to tRNA with methionine.
3. **Elongation**: Subsequent amino acids are added by translating one codon at a time.
4. **Ribosomes** attach each amino acid to growing protein chain by formation of peptide bonds.
5. **Termination**: When a stop codon is reached, translation stops, and ribosome-mRNA complex falls apart.

**Universal Genetic Code**

<table>
<thead>
<tr>
<th>Codon</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUG</td>
<td>Methionine</td>
</tr>
<tr>
<td>UAA, UGA, UAG</td>
<td>Stop</td>
</tr>
<tr>
<td>UUC, UUA, UUG</td>
<td>Leucine</td>
</tr>
<tr>
<td>UAC, UAU</td>
<td>Threonine</td>
</tr>
<tr>
<td>UAA, UGA, UAG</td>
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</tbody>
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Translation: Initiation at Start Codon

Translation: During Elongation one Amino Acid is Added at a Time

Elongation: Ribosome Travels Down mRNA, Reading One Codon at a Time

Termination: Once Stop Codon is Reached, Complex Disassembles

Regulation of Bacterial Gene Expression
- Protein expression requires large amounts of energy.
- Cell saves energy by only making necessary proteins.
  - Constitutive genes: Products made constantly by the cell, synthesis is not regulated. 60-80% of genes. Example: Genes for enzymes of major metabolic pathways.
  - Regulated genes: Products made only when needed by cell. Synthesis is tightly regulated. 20-40% of genes.

Regulation of Bacterial Gene Expression
- Repression
  - Inhibits gene expression and decreases enzyme synthesis.
  - Response to overabundance of a metabolic pathway product.
  - Repressors block RNA polymerase from transcribing gene(s).
- Induction
  - Turns on gene transcription.
  - Inducers stimulate transcription of gene(s) by RNA polymerase.
  - Example: Lac operon
Operon Model of Gene Expression

Operon: Group of metabolically related genes that are transcribed together and a control region that regulates their transcription as a unit.

Contains:
- Structural genes: Code for protein products.
- Promoter: Site where RNA polymerase initiates transcription.
- Operator: DNA segment that controls passage of RNA polymerase.
- Outside of operon:
  - Repressor gene: Codes for repressor protein that blocks operon transcription.

Mutation: Change in Genetic Material

Mutation: Change in the nucleotide sequence of DNA.

These changes may be harmful, beneficial, or have no effect (neutral) on the individual or cell.

Silent mutations: Do not affect activity of gene product.

May or may not change amino acid sequence.

Spontaneous mutations: Occur spontaneously during replication.

Error Rate: Low. In bacteria 1 out of $10^{10}$ bases is mutated during replication. *E. coli* has $4 \times 10^9$ bases, resulting in less than one mutation per replication.

DNA polymerase has proofreading mechanism.

Mutagens: Many chemicals, X rays, ultraviolet light, and other forms of radiation can cause mutations. Increase mutation rate by a factor of 10 to 1000.

Types of Mutations

- Base Substitution (Point Mutation): Single nucleotide is replaced with a different base. After replication, base pair changes.
  - Missense mutation: Results in amino acid substitution.
    - Example: Sickle cell anemia.
  - Nonsense mutation: Creates a stop codon which truncates protein. Only a fragment is synthesized.
  - Silent mutation: Protein sequence and/or activity is not altered.

- Frameshift Mutation: Several nucleotides are inserted or deleted into a gene. These mutations may shift the reading frame of translation, resulting in a completely different amino acid sequence after mutation site.

Genetic Transfer and Recombination

Genetic Recombination: Exchange of genes between two DNA molecules to form new combinations of genes on a chromosome.

Genetic recombination contributes to an organism’s genetic diversity.

In eucaryotes recombination occurs during meiosis through a process called crossing over.

In procaryotes there are several different mechanisms of genetic recombination: Transformation, conjugation, and transduction.

In all cases, it involves a DNA donor and a DNA recipient cell.

Recombination occurs in a small percentage of a bacterial population.

Transformation of Bacteria in Griffith’s Experiment

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Transformation in Bacteria

- Genes are transferred from one bacterial cell to another in the form of naked DNA.
- Initial work done in 1928 by Frederick Griffith on two strains of *Streptococcus pneumoniae*.
  - Smooth strain: Caused disease due to capsule.
  - Rough strain: Did not cause disease.
- Experiments with heat killed smooth bacteria and live rough bacteria, demonstrated the presence of a transforming factor.
- In 1944, Avery and others demonstrated that transforming material was indeed DNA. This was important in establishing that genetic material was DNA.

Transformation of Bacteria in Griffith’s Experiment

![Transformation of bacteria](https://example.com/fig151.jpg)
Transformation: Cells Take up Naked DNA

Only a small percentage of donor DNA is transferred.

Transformation occurs naturally in some bacteria (Bacillus, Neisseria, Hemophilus, Streptococcus, and Staphylococcus).

Other cells can be chemically treated to accept foreign DNA (competent cells). Example: E. coli.

Conjugation in Bacteria

- Genetic material is transferred from one bacterial cell to another through direct contact.
- Gram negative cells form sex pili.
- Gram positive cells produce sticky surface molecules.
- Requires fairly high cell density.

Conjugation Requires Cell to Cell Contact

Transduction in Bacteria

- Genetic material is transferred from one bacterial cell to another through a virus (bacteriophage).
- Transduction may be generalized or specialized.
- Many genes for toxins are transferred by specialized transduction:
  - E. coli O157:H7: Shiga-like toxin.
  - Corynebacterium diphtheriae: Diphtheria toxin.
  - Streptococcus pyogenes: Erythrogenic toxin.

Transduction: Bacteriophage Transfers DNA From One Cell to Another

- A phage injects the donor bacterial cell.
- Phage DNA and proteins are made, and the bacterial chromosome is broken down into pieces.
- Occasionally during phage assembly, pieces of bacterial DNA are packaged in a phage head. These released phage particles containing bacterial DNA.
- A phage carrying bacterial DNA enters a new host cell, the recipient cell.
- Recombination can occur, producing a recombinant cell with a genome different from either parent and recipient cells.