Chapter 9

Topics
- Genetics
- Flow of Genetics/Information

**Genetics**

- **Genome** - the sum total of genetic information in a organism
- **Genotype** - the A's, T's, G's and C's
- **Phenotype** - the physical characteristics that are encoded within the genome

Examples of Eukaryotic and Prokaryotic Genomes

**Chromosome**

- Prokaryotic (E. coli ~ 4,288 genes)
  - 1 circular chromosome ± extrachromosomal DNA (plasmids)
- Eukaryotic (humans ~ 20 -25,000 genes)
  - Many paired chromosomes ± extrachromosomal DNA (Mitochondria or Chloroplast)
  - Subdivided into basic informational packets called genes

Genes

- **Three categories**
  - **Structural** - genes that code for proteins
  - **Regulatory** - genes that control gene expression
  - **Encode for RNA** - non-mRNA

Flow of Genetics/Information

**The Central Dogma**

- **DNA ➔ RNA ➔ Protein**

  - **Replication** - copy DNA
  - **Transcription** - make mRNA
  - **Translation** - make protein
DNA

- Structure
- Replication
- Universal Code & Codons

Escherichia coli with its emptied genome!

Structure

- Nucleotide
  - Phosphate
  - Deoxyribose sugar
  - Nitrogenous base
- Double stranded helix
  - Antiparallel arrangement

Nitrogenous bases

- Purines
  - Adenine
  - Guanine
- Pyrimidines
  - Thymine
  - Cytosine

Versions of the DNA double helix
**Replication**

- Semiconservative - starts at the **Origin of Replication**
- **Enzymes**
  - Helicase
  - Dna Pol III
  - DNA Pol I
  - Primase
  - Gyrase
  - Ligase
- Leading strand
- Lagging strand
  - Okazaki fragments

**Semiconservative**

- New strands are synthesized in **5’ to 3’** direction
- Mediated by DNA polymerase III- only works in **5’ to 3’** direction

**The function of important enzymes involved in DNA replication**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helicase</td>
<td>Unzipping the DNA helix</td>
</tr>
<tr>
<td>Primase</td>
<td>Synthesizing an RNA primer</td>
</tr>
<tr>
<td>DNA polymerase III</td>
<td>Adding bases to the new DNA chain; proofreading the chain for mistakes</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>Removing primer, closing gaps, repairing mismatches</td>
</tr>
<tr>
<td>Ligase</td>
<td>Final binding of nicks in DNA during synthesis and repair</td>
</tr>
</tbody>
</table>

**Leading strand**

- RNA primer initiates the **5’ to 3’** synthesis of DNA in **continuous** manner
Lagging strand

- Multiple Okazaki fragments are synthesized
- Okazaki fragments are ligated together to form one continuous strand

Look at the DNA Fork Movie on McGraw Hill Website

Replication processes of plasmids and viruses involve a rolling circle

Transcription is the synthesis of RNA from a DNA template – second step in the central dogma -

RNA is transcribed from DNA

RNA

- Transcription - 3 main types of RNA
  - Message RNA (mRNA)
  - Transfer RNA (tRNA)
  - Ribosomal RNA (rRNA)
- Codon - Remember that in RNA, there are no T's - just U's
rRNA combines with ribosomal proteins to form ribosomes which serve as sites for the assembly of amino acids into proteins.

tRNA – select amino acids and transfer the amino acids to the growing chain of a protein.

mRNA – carries the information for the proteins in the form of codons – one codon/one amino acid

**Codons**
- Triplet code that specifies a given amino acid
- Multiple codes for one amino acid – REDUNDANT or DEGENERATE
- 20 amino acids
- Start codon - AUG
- Stop codons – UAA, UAG, UGA

**mRNA**
- Copy of a structural gene or genes of DNA
  - Can encode for multiple proteins on one message
- Thymidine is replaced by URACIL
- The message contains a codon (three bases)

**The Genetic code - Wow!!!!!**

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Second Base Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAA</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>UAC</td>
<td>Valine</td>
</tr>
<tr>
<td>GUA</td>
<td>Asparagine</td>
</tr>
<tr>
<td>UAU</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>UAU</td>
<td>Urea</td>
</tr>
<tr>
<td>UAG</td>
<td>Threonine</td>
</tr>
<tr>
<td>CUA</td>
<td>Leucine</td>
</tr>
<tr>
<td>CAC</td>
<td>Histidine</td>
</tr>
<tr>
<td>CCA</td>
<td>Lysine</td>
</tr>
<tr>
<td>AUA</td>
<td>Methionine</td>
</tr>
<tr>
<td>AAG</td>
<td>Arginine</td>
</tr>
<tr>
<td>AGA</td>
<td>Arginine</td>
</tr>
<tr>
<td>GUA</td>
<td>Valine</td>
</tr>
<tr>
<td>GAC</td>
<td>Asparagine</td>
</tr>
<tr>
<td>GCA</td>
<td>Glnucleic</td>
</tr>
<tr>
<td>GAA</td>
<td>Glutamic acid</td>
</tr>
</tbody>
</table>

The polymerase continues transcribing until it reaches a termination site and the mRNA transcript is stopped for translation. Note that the section of the DNA that has been transcribed is removed from its original configuration.
**Transcription**

- RNA Pol
- Template strand (3' → 5')
- Newly made mRNA (5' → 3')
- Promoter – binding site for RNA Pol
- Average size for mRNA – 1200 bases

**Fig. 9.15 Interpreting the DNA code**

**Translation**

- Translation
  - Protein synthesis have the following participants
    - mRNA
    - tRNA with attached amino acid - "loaded" tRNA
    - Ribosome

**The “players” in translation**

**Relationship between tRNA and mRNA**

For procaryotes, translation can occur at multiple sites on the mRNA while the message is still being transcribed.
Transcription in Prokaryotes and Eukaryotes

- 1 mRNA = 1 protein
- 1 mRNA = several proteins (polycistronic)
- Different compartments for each event
- Presence of introns

Regulation

- Lactose operon (INDUCIBLE) - genetic induction
  – Utilize lactose as a food source
- Repressible operon - genetic repression
  – Amino acids, nucleotides

Lactose Operon – Turned OFF - Glucose

- If Operon Off, in the absence of lactose, a repressor protein (the product of a regulatory gene located elsewhere on the bacterial chromosome) attaches to the operator of the operon. This effectively blocks the operator and prevents any transcription of structural genes downstream (to its right). Suppression of transcription (and consequently, of translation) prevents the unnecessary synthesis of enzymes for processing lactose.

Lactose Operon – Turned ON - No Glucose

- If Operon On, upon entering the cell, the glucose molecule induces a genetic inducer by binding to the repressor, which then moves away from the operator. The RNA polymerase then has free access to bind to the promoter and initiate transcription, and the enzymes produced by translation of the mRNA perform the necessary functions of using lactose substrates.

The Arg Operon - responsible for synthesizing the amino acid ARGinine

EX. of REPRESSION
**Arginine Operon – Turned ON – Need to Make Arg**

- RNA polymerase
- Transcription
- Repressor is inactive (wrong shape to attach to operator)
- Enzymes synthesize arginine
- Arginine immediately used in metabolism

**Arginine Operon – Turned OFF – NO Need to Make Arg**

- Repressor is active (correct shape to bind to operator)
- The repression complex affects the transcription and blocks the RNA polymerase from transcribing genes for arginine synthesis.

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**Comparison of Induction and Repression**

<table>
<thead>
<tr>
<th>Regulatory Mechanism</th>
<th>Type of Pathway Regulated</th>
<th>Regulating Substance</th>
<th>Condition Leading to Gene Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction (lac operon)</td>
<td>Catabolic</td>
<td>Nutrient (Lactose)</td>
<td>Presence of Nutrient</td>
</tr>
<tr>
<td>Repression (arg operon)</td>
<td>Anabolic</td>
<td>End product (arginine)</td>
<td>Absence of End Product</td>
</tr>
</tbody>
</table>

**Mutations**

- Changes made to the DNA - two main types
  - Spontaneous – random change
  - Induced – chemical, radiation

**Specific examples of mutations**

- **Point** – change a single base
- **Nonsense** – change a normal codon into a stop codon
- **Frameshift** – reading frame of the mRNA changes

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**Point mutations** are a change in a single base – the reading frame is **not** affected, but the mutation may be either expressed or silent.

**Frame-shift mutations** are the deletion or addition of one or more bases. These mutations change the reading frame of all downstream codons.
Spontaneous mutations are mutations that are caused by errors in the synthesis of DNA. Errors occur at the rate of 1 error every $10^3$ or $10^4$ nucleotides.

However, most organisms, both pro and eukaryotic, possess repair systems that lower the frequency of errors to one error in $10^9$ to $10^{11}$ nucleotides.

Prokaryotes have repair systems that can repair damaged DNA. **Light repair** of DNA (photoreactivation) can repair thymine dimers induced by UV light.

**Dark repair** can identify and excise defective DNA and replace the defective DNA with the correct sequence based on the template strand.

**Eukaryotes have similar systems**

Xeroderma pigmentosa is a genetic disease of humans that is due to an inherited defect in DNA repair.

Exposure to sun (UV light) results in a dramatically increased rate of skin cancer due to UV induced mutation of DNA in the skin cells.

Xeroderma pigmentosa

Genetic disease where DNA repair process is damaged - patients lack DNA photolyase

Results in multiple skin cancers
The Ames test is used to screen environmental and dietary chemicals for mutagenicity and carcinogenicity without using animal studies.

Fig. 9.22 The Ames test.

### Transformation – free DNA

- Nonspecific acceptance of free DNA by the cell (ex. DNA fragments, plasmids)
- DNA can be inserted into the chromosome
- Competent cells readily accept DNA

**Mechanism of Transformation**

'Naked' DNA taken up by competent cell.

The DNA is free in the extracellular space. Cells are only competent to receive the DNA at certain periods of the life cycle.

A competence factor is released by the cell and facilitates the entry of the DNA.

The amount of DNA that enters is small - less than 5% of the cell's genome.

**Recombination**

- Sharing or recombining parts of their genome
  - Conjugation
  - Transformation
  - Transduction

DNA released from a killed cell can be accepted by a live competent cell, expressing a new phenotype.

Fig. 9.24 Griffith's classic experiment in transformation

To successfully transform cells, the DNA must be recombined into the recipient cell's genomic material.

In recombinant DNA work, cells can be made "competent" to receive DNA. Then the recipient cells can be readily transformed.

Not all bacteria are subject to transformation - natural or induced.
**Transduction THINK BACTERIOPHAGE**

- Bacteriophage infect host cells
- Serve as the carrier of DNA from a donor cell to a recipient cell
  - Generalized
  - Specialized

A phage infects a bacterium and “chooses” a **lytic** cycle or **lysogenic** cycle - Figure 8.3.

If **lysogenic cycle** is chosen, the phage genome recombines into the bacterial genome and becomes a prophage.

Lysogenic phages are **specialized transducing phages** and can transduce only specific regions of the bacterial genome.
Specialized and General Transduction

Generalized transducing phages undergo a lytic cycle and are capable of transducing any part of the donor’s genetic information.

Transduction is significant

The ability of a lysogenic phage to recombine into a bacterial genome suggests a parallel evolution of phage and bacteria since there must be sequence similarities at the site of integration.

Transduction is a mechanism to transfer genetic material from one cell to a second.

Fig. 9.27 Specialized transduction
Conjugation

• Transfer of plasmid DNA from a F+ (F factor) cell to a F- cell
• An F+ bacterium possesses a pilus
• Pilus attaches to the recipient cell and creates pore for the transfer DNA
• High frequency recombination (Hfr) donors contain the F factor in the chromosome

Lederberg discovered conjugation in 1946

Mechanism of conjugation

In one type of conjugation, the population of cells capable of conjugating contain two types of cells F+ and F-; the former are the donor cells and the latter are the recipient cells. The donor cells have an F plasmid—sex pili and DNA Transfer. Conjugation in this case is a transfer of the F plasmid from the donor to the recipient.

The F plasmid codes for the synthesis of pili which are instrumental in the formation of the conjugal bridge & DNA Transfer

A second type of conjugation is F+ to Hfr conversion.

A third type of conjugation is F' plasmids are created when the Hfr plasmid recombines out of the bacterial genome imprecisely and carries with it a segment of the bacterial genome. That segment can the be transferred to a recipient cell as in F+ conjugation.

(d) High-frequency (Hfr) transfer involves transmission of chromosomal genes from a donor cell to a recipient cell. The donor chromosome is duplicated and transmitted in part to a recipient cell, where it is integrated into the chromosome.
**Significance of conjugation**

In Hfr conjugation significant amounts of genetic material may be transferred.

Genetic information including determinants of pathogenicity or antimicrobial resistance may be transferred cell to cell.

Hfr conjugation is an excellent procedure to map genes of conjugable bacteria.

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**The mechanisms of gene transfer are summarized –**

<table>
<thead>
<tr>
<th>Kind of transfer</th>
<th>Summary of the effects of various transfers of genetic information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transformation</strong></td>
<td>Transfers less than 1 percent of cell's DNA. Requires competence factor. Changes certain characteristics of an organism depending on which genes are transferred.</td>
</tr>
<tr>
<td><strong>Transduction</strong></td>
<td>Transfer is effected by a bacteriophage.</td>
</tr>
<tr>
<td>Specialized</td>
<td>Only genes near the prophage are transferred to another bacterium.</td>
</tr>
<tr>
<td>Generalized</td>
<td>Fragments of host bacterial DNA of variable length and number are packed into the head of a virus.</td>
</tr>
<tr>
<td><strong>Conjugation</strong></td>
<td>Transfer is effected by a plasmid.</td>
</tr>
<tr>
<td>F'</td>
<td>A single plasmid is transferred.</td>
</tr>
<tr>
<td>Hfr</td>
<td>An initiating segment of a plasmid and a linear sequence of bacterial DNA that follows the initiating segment are transferred.</td>
</tr>
<tr>
<td>F''</td>
<td>A plasmid and whatever bacterial genes adhere to it when it leaves a bacterium are transferred.</td>
</tr>
</tbody>
</table>