Course goal:

To provide you with the *basic principles* of microbiology and to relate the importance of these principles to the actual host/microbial interrelationships that you may encounter in your career. Emphasis will be placed on understanding the *mechanisms* involved in mediating oral and ocular health and disease.
**DNA: THE GENETIC MATERIAL**

**Chromosome:**
Most bacteria have one circular DNA chromosome ranging in size from 1,000 to 8,000 kilobase pairs (kb).

**Bacterial Genome:** The *collection of all the genes* present on the bacteria's chromosome or its extrachromosomal genetic elements.

- *Plasmid* = Extrachromosomal genetic element
- *Genomes* contain *operons*
- *Operons* are made up of *genes*
- *Promoters and operators* control genes

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**CONTENT**

- DNA: The genetic material
- Replication of DNA
- Transcriptional control
- Mutation, repair and recombination
- Gene exchange
- Genetic transfer
- Genetic engineering

**Plasmid**

- Tet 
- Amp 
- Ori 
- pBR322
- 4361bp

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Nucleic Acid… Where is it?

DNA Structure

The two strands are held together by hydrogen bonds

purines

Adenine

Guanine

pyrimidines

Thymine

Cytosine
DNA REPLICATION

- Replication initiated at the oriC
- Requires many enzymes (helicase, primase etc)
- New DNA is synthesized semiconservatively* 
- DNA synthesis proceeds bidirectionally

*New DNA is synthesized semiconservatively, meaning each new DNA strand is a mix of old and new bases.
DNA duplication in the lab – The Polymerase Chain Reaction (PCR)

1. Extract DNA for amplification

   - Oligonucleotide primers specific for target DNA fragment,
   - Thermo stable DNA polymerase
   - Nucleotide triphosphates

2. Denature DNA template (95°C)

3. Anneal oligonucleotide primers (40 – 65°C)

4. Synthesize second strand with Taq DNA polymerase (72°C)

   Repeat as many cycles as required

Semi-conservative replication
- One strand from parent
- One strand newly synthesized
Transcriptional control

i. Basic Prokaryotic gene organization
ii. The lac operon
iii. Induction of the lac operon
iv. Positive control of the lac operon
v. Two-component systems in bacteria

Central dogma: DNA $\rightarrow$ RNA $\rightarrow$ Protein

![Diagram of the central dogma]

- DNA $\rightarrow$ Transcription $\rightarrow$ mRNA $\rightarrow$ Translation $\rightarrow$ Protein
i. Basic Prokaryotic Gene Organization

Promoter properties

CONSENSUS SEQUENCES

TTGACA \text{---------}N_{17}\text{---------}TATAAT\text{---------}N_{5\text{-}9}\text{---------}A/G

5'\text{-TTGACA}\text{NNNNNNNNNNNNNNNNNNNNNNN(T/C)NNNNNN-}\
3'\text{-AACTGNNNNNNNNNNNNNNATTTANNNNNNNNN(T/C)NNNNNN------}
ii. The lac operon (negative regulation)

- Repressor protein is produced
- Binds to the operator site (O) adjacent to the promoter
- Repressor competes with RNAP at the operator site
- Transcription of the structural genes is blocked
iii. Induction of the lac operon

- When lactose is present, the lac genes can be expressed; lactose can then be utilized by the cell.

iv. Positive control of the lac operon

CAP = catabolite gene-activator protein
In sum…

**Negative control:**
Genes are expressed unless they are switched off by a *repressor* protein

**Positive control:**
Are not transcribed until they switched on by an *active regulator protein* (apo-inducer)

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vi. **Two-component systems in bacteria**

- Regulatory system that ties the expression of specific genes to chemical signals in their environment.
- Two components - one that senses and a second that transmits the signal.

![Diagram of a two-component system](image)

**Histidine kinase (HK) + ATP → P-HK + ADP**

**P-HK + response regulator (RR) → HK + P-RR**

- pH, NaCl, K⁺
- Temperature
- Iron, Mg²⁺
- NO, CO, O₂
Mutation, Repair and Recombination

i. Single point mutations
ii. DNA repair mechanisms
iii. General excision repair
iv. Silent mutations

General …

**Mutation:**
• Heritable change in nucleotide sequence

**Point mutations:**
• Single base change

**Other:**
• Deletions, insertions, duplications or inversions of DNA bases

*Mutation rate:* probability that any one cell will mutate during the period of time required by a cell to divide to form a new generation of cells:
i. Single Point Mutations:

Transition: Purine replaced with a purine (A with a G; G with an A)
Pyrimidine replaced with a pyrimidine (T with a C; C with a T)

\[
\begin{align*}
\text{A} & \quad \rightarrow \quad \text{G} \\
\text{T} & \quad \rightarrow \quad \text{C}
\end{align*}
\]

Transversion: Purine replaced with a pyrimidine or visa versa

\[
\begin{align*}
\text{G} & \quad \rightarrow \quad \text{T} \\
\text{T} & \quad \rightarrow \quad \text{G}
\end{align*}
\]

- Exposure of DNA to UV light results in the formation of thymine dimers.
- Cannot act as template for DNA polymerase
ii. DNA Repair Mechanisms

- **Direct DNA repair**: enzymatic removal and replacement of damaged base

- **Excision repair**: excision of DNA section that contains the damage followed by synthesis of new DNA strand

- **Post-replication repair**: if both strands of DNA are damaged; genetic information is retrieved from the sister strand base sequence and recombined back into place.

- **SOS repair**: Inducible after damage occurs, many enzymes are induced. Requires recA gene that codes for a recombination protein (RecA).

- **Error prone repair**: a *LAST RESORT* system. It’s better to have mutations left over from repair rather than not to be able to survive at all.

iii. General excision repair

1. Damaged base recognized by endonuclease, cuts phosphodiester on both sides of damage
2. Exonuclease carry out excision of DNA fragment with damaged base
3. DNA polymerase I fills out the gap from the free 3'-OH
4. DNA ligase seals the nick between newly synthesized fragment and original DNA strand.
iv. Silent mutations

Gene Exchange in Prokaryotic Cells

Exchange of DNA between cells may provide functions that are useful between bacteria (e.g. antibiotic resistance markers).

Three mechanisms:
1. Transformation – uptake of naked DNA

2. Transduction – bacteriophage-mediated DNA transfer (transposons?)

3. Conjugation – bacterial mating
Transformation

Acquisition of new genetic markers by incorporating exogenous of foreign DNA

Transduction

Transfer of genetic information from one bacterium to another by a bacteriophage

Conjugation

Mating or quasisexual exchange of genetic information from one bacterium (donor) to another (recipient)
Plasmids contain genetic information that can be exchanged

Bacteriophages

- **Bacteriophage**: Virus that infect bacteria;
- Are not cellular; cannot grow outside the bacteria;
- Protein *outer shell* called **CAPSID** (some may have lipid with shell);
- May have *tails* or be *tailless*; may be *filamentous*;
- Nucleic acid: *DNA* or *RNA*, single or double stranded
**Lytic Life Cycle**

- Infection of sensitive bacterium
- Phage DNA becomes integrated into bacterial chromosome
- Bacterium multiplies
- Individual cell produces phage components
- Cell lyses and release of mature phage particles

**Lysogenic life cycle** - phage DNA replicate with bacterial chromosome

**Generalized Transduction:**

A virus carries **random** DNA fragments from donor to recipient bacterium.

**Specialized transduction:**

A virus carries **specific** genes from a donor bacterium to a recipient bacterium.
**Transposons**

**Definition:** Mobile genetic elements that can transfer DNA within a cell from one position to another in the genome or between different molecules of DNA (e.g., plasmid to plasmid or plasmid to chromosome)

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**Transposition**

Gene B is now “disrupted”
Tn3 – member of the TnA transposon family

Inverted repeat (IR) Res site

TnpA – transposase
TnpR – resolvase
β-lactamase – antibiotic resistance marker
Inverted repeat – 38 base pairs

Chromosomal DNA can be exchanged...
Important - Plasmids contribute to the evolution of antibiotic resistant bacteria…

Genetic engineering

Commercial production of:
- Therapeutic products
- Diagnostic products
- Agricultural products
- Chemical products
- Other

Recombinant cells produce:
- Human interferon
- Human insulin
- Human growth hormone
- Interleukin-2
- Tumor necrosis factor
- Tissue plasminogen activator
- Erythropoietin

Genetically engineered microorganisms can be patented!
Restriction enzymes:

GAATTC
CTTAAG

EcoR1

G
AATTC
CTTAAG

or

CCCGGG
GGGCCC

SmaI

CCC
GGG
CCC
Recent advances and applications

- HT sequencing
- Microarrays
- PCR
HT Sequencing

• > 600 microbial species inhabiting the human oral cavity;
• ~½ have been successfully characterized;
• Current (2008) solution: Sequence everything!!!

Technologies:
1. Nanopore
2. Pyrosequencing
• 454 Life Sciences: sequenced 25 million bases, at 99% or better accuracy, in one four-hour run (assembled the Mycoplasma genitalium genome with 96% coverage at 99.96% accuracy in one run).

DNA microarrays (MA): Implications for Dentistry and Oral Diagnostics

• Study cancers and infectious diseases of the oral cavity;
• NIH funded TIGR to generate MA’s for S. mutans and P. gingivalis;
• MA technology will allow dentists to “fingerprint” a patients oral cavity and monitor changes;
Benefits of Recombinant DNA Technology

Production of Biological and Medical Products:
- Insulin, growth hormones, interferon's, anti-clotting factors etc

Agriculture:
- Generation of disease resistant plants

Laboratory and Forensic Diagnostics:
- Recombinant molecules used in assay kits
- Paternal suits
- Genetic testing and counseling

Research:
- Indispensable tool for discovery