Chapter 7: Microbial Genetics

1. DNA Replication
2. Gene Expression
3. Gene Regulation
4. Mutations
5. DNA Recombination & Gene Transfer
1. DNA Replication

Chapter Reading – pp. 197-198, 201-205
base-pairing strands are always anti-parallel
Elongation of Nucleotide Polymers

DNA (like RNA) is a polymer of nucleotides:

- Each nucleotide to be added is a nucleotide triphosphate (dNTP)
- Energy for the *endergonic* synthesis of DNA comes from the release of diphosphate from each dNTP!
- Nucleotides can only be added to the 3’ end (synthesis is 5’ to 3’)

- Guanosine triphosphate deoxyribonucleotide (dGTP)
- Guanine nucleotide (dGMP)
- Guanine base

Deoxyribose

## (a)

-existing DNA strand + triphosphate nucleotide

Diphosphate released, energy used for synthesis

-longer DNA strand

## (b)

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Initiation of DNA Replication

Replication begins at an **origin of replication** (Ori):

- initiation factor proteins assemble at Ori DNA sequence
- DNA helicase unwinds the DNA (in both directions)
- stabilizing proteins keep strands separated, allowing DNA synthesis to begin…

Chromosomal proteins (histones in eukaryotes and archaea) removed

**Diagram:**
- DNA helicase
- Replication fork
- DNA polymerase III
- Stabilizing proteins

(a) Initial processes

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Leading Strand Synthesis

DNA synthesis always begins with an RNA primer:

- the DNA-synthesizing enzyme DNA polymerase can only add nucleotides to an existing strand

- Primase produces a short, complementary RNA primer at the Ori

- DNA polymerase extends primer of leading strand in direction of fork…

(b) Synthesis of leading strand
Because DNA synthesis must be 5’ to 3’, lagging strand synthesis proceeds away from the replication fork:

- multiple strands must be primed and extended as the fork unwinds
- another type of DNA polymerase replaces RNA primers with DNA, and DNA ligase stitches the pieces together
Summary of DNA Replication

Both strands serve as a template:

- synthesis is always 5’-3’
- *leading* strand synthesis is continuous, *lagging* strand synthesis is discontinuous

Each new DNA fragment requires an RNA primer:

- DNA synthesis cannot begin without a primer to add to

Some important enzymes:

**DNA Polymerase** (synthesizes new DNA)

**Helicase** (unwinds DNA)  **Primase** (makes RNA primers)

**DNA Ligase** (“stitches” fragments together)
DNA Replication in Prokaryotes

Prokaryotic DNA replication proceeds in both directions from the Origin until the 2 forks meet and the new copies are enzymatically separated from each other.

- eukaryotic chromosomes are linear which requires special enzymes to complete replication of the ends, and they also have multiple origins of replication due to the immense length of ea DNA molecule
2. Gene Expression

Chapter Reading – pp. 206-216
Gene Expression

The expression of a gene into a protein occurs by:

1) **Transcription** of a gene into RNA
   - produces an RNA copy of the coding region of a gene
   - the RNA transcript may be the actual gene product (rRNA, tRNA) or be translated into a polypeptide gene product (mRNA)

2) **Translation** of mRNA transcript into polypeptide
   - accomplished by **ribosomes** with the help of tRNA
**DNA (genotype)**

```
5'  A T G C G G G T A C T T A 3'
3'  T A C G C C C A T G A A T 5'
```

**TRANSCRIPTION**

```
5'  A U G C G G G U A C U U U A 3'
3'  A U G C G G G U A C U U U A 5'
```

**TRANSLATION (by ribosomes)**

- Methionine
- Arginine
- Tyrosine
- Leucine

... Polypeptide

**PHENOTYPE**
1) Initiation

- **RNA polymerase** binds to the **promoter** sequence of gene
  - promoter recognition accomplished via transcription factor proteins such as the “Sigma factors” in bacteria
- promoter serves to target and orient RNA polymerase
- once “docked” at promoter, RNA polymerase unzips DNA
Elongation of the RNA Transcript

2) Elongation

• RNA polymerase synthesizes a complementary RNA strand

• only 1 DNA strand is used as a template, the other strand is referred to as the “coding strand”

• ribonucleotides (rNTPs) can only be added to the 3’ end of an RNA transcript, thus elongation is in a 5’→3’ direction

After triphosphate ribonucleotides align with their DNA complements, RNA polymerase links them together, synthesizing RNA. The triphosphate ribonucleotides also provide the energy required for RNA synthesis. No primer is needed.
In self-termination, the transcription of DNA terminator sequences cause the RNA to fold, loosening the grip of RNA polymerase on the DNA.

In enzyme-dependent termination, a termination enzyme pushes between RNA polymerase and the DNA, releasing the polymerase.

- triggered by stem/loop structure in RNA or termination factors such as the Rho protein
Concurrent Transcription of DNA

A given gene can be transcribed concurrently by multiple RNA polymerases:

- can yield many rapidly produced RNA copies of a gene
Prokaryotes typically have several functionally related genes arranged in tandem groups called operons:

- multiple genes under the control of a single promoter that are contained in a single RNA transcript
RNA Splicing in Eukaryotes

Unlike prokaryotic RNA transcripts, eukaryotic transcripts need to be processed:

- a special 5’ cap structure must be added
- a poly-A tail is added
- introns are spliced out so that the remaining exons form a continuous coding sequence
Various Roles of RNA Transcripts

1) messenger RNA (mRNA)
   - RNA copy of a gene that encodes a polypeptide

2) ribosomal RNA (rRNA)
   - RNA that is a structural component of ribosomes

3) transfer RNA (tRNA)
   - delivery of “correct” amino acids to ribosomes during translation

For some genes, the end-product is the RNA itself (rRNA, tRNA)
The Genetic Code

If the DNA sequence is: CATGCTGGGGAATAG

(transcription)

The mRNA copy is: CAUGCCUGGGAATAUG

(translation)

The polypeptide is: *Met-Pro-Gly-Gln-(stop)

all proteins begin w/Met
Overview of Translation

The building of a polypeptide, 1 amino acid at a time, by ribosomes using info in mRNA:

- ribosomes bind directly to mRNA, “read” codon by codon
  - ribosomes always start at AUG (methionine)
- translation also involves tRNAs, each of which is attached to 1 of the 20 amino acids (AAs)
  - ribosomes match the right tRNA (via anticodon) with the right codon in the mRNA, then add its AA to the growing protein
Prokaryotic vs Eukaryotic Ribosomes

(a) Prokaryotic ribosomes:
- 5S rRNA + 34 polypeptides → 50S subunit
- 23S rRNA
- 16S rRNA + 21 polypeptides → 30S subunit

(b) Eukaryotic ribosomes:
- 5S rRNA + 5.8S rRNA + 28S rRNA + 18S rRNA + > 33 polypeptides → 60S subunit
- 60S subunit + 49 polypeptides → 80S ribosome
tRNA Structure & Function

- Acceptor stem
- Hydrogen bonds
- Anticodon

(a) Anticodon
(b) tRNA icon

amino acid
1. Specific sequences in the rRNA of the small ribosomal subunit align (base pair) with complementary sequences near the start codon (AUG) in the mRNA

2. Anticodon of initiator tRNA carrying MET (eukaryotes, archaea) or fMET (bacteria) base pairs with start codon

3. Joins with the large ribosomal subunit to complete the initiation complex with tRNA^{MET} in P-site, A-site empty
Elongation of Translation

Translation terminates when a stop codon is reached

Movement of ribosome

Two more cycles

Movement of ribosome

Growing polypeptide
Elongation & Termination of Translation

ELONGATION

4. tRNA with anticodon complementary to codon in A site joins the initiation complex

5. ribosome catalyzes peptide bond formation between amino acids attached to each tRNA

6. ribosome shifts 3 nucleotides (1 codon) on mRNA (in a 5’ to 3’ direction)

7. new tRNA with complementary anticodon enters the A site and the process continues until...

TERMINATION

8. A “stop” codon triggers termination of the process
Transcription & Translation in Prokaryotes

- gene expression is typically not “segregated”
- transcription & translation can occur simultaneously
  - no nuclear membrane separating mRNA from ribosomes
  - no processing of RNA transcripts is required
3. Gene Regulation

Chapter Reading – pp. 216-218
Levels of Gene Regulation

The expression of a gene into functional gene products can be regulated at multiple levels:

1. **TRANSCRIPTION**
   - (regulation of rate at which gene is transcribed)
   - mRNA transcript stability
     - ("half-life" of transcripts)

2. **TRANSLATION**
   - (regulation of translation of mRNA)

   - post-translational modifications
     - (e.g., cleavage of polypeptides, addition of chemical groups)

*key level of regulation
Regulation of Transcription

The focal point is whether or not RNA polymerase binds the promoter of a gene and initiates transcription which depends on:

1) Affinity of RNA polymerase for a given promoter

• some promoters are “strong” and bind RNA polymerase with high affinity

• some promoters are “weak” and bind RNA polymerase with low affinity, requiring help from special proteins called transcription factors

• the strength of a promoter depends on its sequence
2) Influence of proteins collectively referred to as transcription factors

- proteins that help RNA polymerase bind a promoter (commonly referred to as “activators”)

- proteins that inhibit or prevent RNA polymerase from binding a promoter (referred to as “repressors” or “inhibitors”)

The levels of various repressors & activators of transcription depend on the cellular environment, which thus determines which genes are ON or OFF!

Let’s see how this works in genes involved with lactose metabolism in *E. coli*...
The lac operon is a genetic control system in bacteria that regulates the expression of genes involved in lactose metabolism. It consists of a Regulatory gene, a Promoter, and Structural genes.

(a) Lac operon is OFF
- Repressor blocks the Operator, preventing transcription.
- mRNA synthesis is not initiated.

(b) Lac operon is ON
- Inducer (allolactose) binds to the repressor, inactivating it.
- The Operator is no longer blocked.
- Transcription of the Structural genes proceeds.

The lac operon is regulated by the presence of lactose or its metabolite, allolactose. In the absence of lactose, the repressor is active, and the operon is OFF. In the presence of lactose or allolactose, the repressor is inactivated, allowing transcription to proceed, and the operon is ON.
The *lac* operon of *E. coli*

The *lac* operon is a module of 3 genes involved in lactose metabolism, *lacZ*, *lacY* & *lacA*, that are transcribed in a single mRNA from a single promoter.

On either side of the promoter are 2 special sequences, the CAP site which binds the activator CAP, and the Operator which binds the *lac* repressor...
When lactose is absent:

The *lac* repressor protein by default is bound to the operator sequence, thus blocking part of the promoter and preventing RNA polymerase from binding and initiating transcription of the *lacZ*, *lacY* & *lacA* genes.

The *lac* operon is OFF since there’s no need for these gene products in the absence of lactose.
When lactose is present w/glucose:

Lactose binds to the *lac* repressor, inducing a change in shape that prevents its binding the Operator sequence.

- with the operator no longer occupied, RNA polymerase can bind promoter & initiate a low level of transcription

Since glucose (a preferred energy source) is present, the *lac* operon is ON “low”.

Lactose binds to the *lac* repressor, inducing a change in shape that prevents its binding the Operator sequence.
When lactose is present w/o glucose:

The *lac* repressor is bound by lactose and inactive, and the low glucose levels activate CAP, a transcriptional activator, which binds the CAP site & enhances binding of RNA polymerase to the promoter.

Since lactose is a much more important source of energy in the absence of glucose, the *lac* operon is ON “high”.

(c)

\[+ \text{lactose} \]

\[- \text{glucose} \ (\text{high cAMP})\]
Summary of the \textit{lac} operon

The \textit{lac} repressor inhibits transcription in the absence of lactose.

The \textit{CAP} protein activates (enhances) transcription when glucose is unavailable.
4. Mutations

Chapter Reading – pp. 220-222
Mutations

A mutation is *any* change in DNA sequence:

- change of one nucleotide to another
- insertion or deletion of nucleotides or DNA fragments
- inversion or recombination of DNA fragments

What causes mutations?

- errors in DNA replication or DNA repair
- chemical mutagenesis
- high energy electromagnetic radiation
  - UV light, X-rays, gamma rays
Effects of Mutations

- Insertions & deletions can cause "frame shifts"

(a) Base-pair substitutions

- Silent mutation: A instead of G
  - Normal sequence: AUG CAG GAC CAU CUU UAG
  - Protein: Met Gln Thr Ser stop
- Missense mutation: A instead of C
  - Normal sequence: AUG GAA GAC CAU CUU UAG
  - Protein: Met Lys Thr Ser stop
- Nonsense mutation: U instead of C
  - Normal sequence: AUG UAG GAC CAU CUU UAG
  - Protein: Met stop

(b) Deletion/insertion

- Deletion: Loss of A
  - Normal sequence: AUG CAG GAC CAU CUU UAG
  - Protein: Met Gln His Leu
- Insertion: G inserted
  - Normal sequence: AUG GAC GAC CAU CUU UAG
  - Protein: Met Ala Asp Ile Leu
Types of Mutations

Silent mutations:
• have no effect on amino acid specification

Missense mutations:
• result in the change of a single amino acid

Nonsense mutations:
• convert a codon specifying an amino acid to a stop codon
  • results in premature truncation of a protein

Insertion/deletion mutations:
• cause a shift in the reading frame of the gene
  • all codons downstream of insertion/deletion will be incorrect
5. DNA Recombination & Gene Transfer

Chapter Reading – pp. 227-232
Horizontal vs Vertical Gene Transfer

**Vertical**
- transfer to the next generation

**Horizontal (or lateral)**
- transfer within the same generation
Homologous Recombination

Unless transferred DNA is circular w/Ori (plasmid), it must recombine with host DNA to be retained.

Recombination can occur between *homologous* (similar) DNA sequences:

- DNA with “same” genes
- facilitated by special proteins
- original DNA is lost
Methods of Gene Transfer

Bacteria can acquire DNA (i.e., new genes) in 3 basic ways:

1) Transformation
   • uptake and retention of external DNA molecules

2) Conjugation
   • direct transfer of DNA from one bacterium to another

3) Transduction
   • the transfer of DNA between bacteria by a virus
Under the right conditions, bacteria can “take in” external DNA fragments (or plasmids) by transformation.

- DNA binding proteins transfer external DNA across cell envelope
- homologous recombination can then occur
- bacterial cells capable of transformation are referred to as competent
Griffith’s Transformation Experiment

**Pathogenic S strain**
- When Griffith injected S strain (encapsulated, pathogenic) cells into the mouse, it developed pneumonia and died.

**Harmless R strain**
- An injection of R strain (unencapsulated, harmless) cells did no harm to the mouse.

**Heat-killed pathogenic cells**
- Furthermore, an injection of heat-killed S strain cells did no harm because the cells were dead.

**Mixed harmless and heat-killed pathogenic cells**
- But when Griffith injected a mixture of live R strain and heat-killed S strain cells into the mouse, it died. When Griffith cultivated the organism from the blood, he found live S strain cells.

**Colonies of pathogenic cells isolated from dead mouse**

**Colonies of harmless cells**

**No colonies isolated from mouse**

**Colonies of harmless and pathogenic cells isolated from dead mouse**
Bacterial Conjugation

Requires an F factor plasmid

- has all “conjugation genes”
- directs formation of a sex pilus
- single DNA strand produced by DNA replication is transferred to F- cell through the sex pilus, recipient produces 2nd strand
Hfr Conjugation

If F factor plasmid is inserted into the host chromosome (Hfr cell), this will result in the transfer of adjacent chromosomal DNA.

- recipient can acquire donor cell genes by recombination
- also useful for mapping the position of bacterial genes

Hfr = “High frequency of recombination”

1. F plasmid integrates into chromosome by recombination.
2. Cells join via a conjugation pilus.
3. Portion of F plasmid partially moves into recipient cell trailing a strand of donor’s DNA.
4. Conjugation ends with pieces of F plasmid and donor DNA in recipient cell; cells synthesize complementary DNA strands.
5. Donor DNA and recipient DNA recombine, making a recombinant F- cell.
Transduction

A virus (phage) particle can transfer DNA fragments from one host cell to another followed by recombination:

- requires a virus to be packaged with bacterial DNA “by mistake”
- infection of another cell by such a virus facilitates the gene transfer followed by homol. recombination
Key Terms for Chapter 7

• transcription factor, activator, repressor
• lac operon, lac repressor, operator, CAP
• leading & lagging strands, primase, DNA ligase, helicase
• missense, nonsense, silent mutations, frame shift
• horizontal vs vertical gene transfer
• homologous recombination
• transformation, transduction, conjugation, Hfr

Relevant Chapter Questions
MC: 1-5, 8, 11-13, 15-20, 22-24
FB: 1-8, 11 SA: 1-3, 5-8, 10-12