Chapter 8: Microbial Genetics

1. Gene Expression
2. Gene Regulation
3. DNA Replication & Mutation
4. Mechanisms of Gene Transfer
1. Gene Expression
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
Overview of Transcription

1. INITIATION: RNA polymerase binds to the promoter sequence, initiating transcription.
2. ELONGATION: RNA polymerase moves along the DNA template, synthesizing a complementary RNA strand.
3. TERMINATION: The RNA polymerase reaches a terminator sequence, signaling the end of transcription.

Promoter sequence
Terminator sequence
Direction of transcription
Template strand of DNA
RNA polymerase
RNA nucleotides
Growing RNA
Completed RNA
Newly made RNA
Transcription is Uni-directional

- ribo-nucleotides can only be added to the 3’ end of an transcript, thus elongation is in a 5’ → 3’ direction
3 Steps of Transcription

1) Initiation

- RNA polymerase binds to the promoter of a gene

- promoter serves to target and orient RNA polymerase
- once “docked” at promoter, RNA polymerase unzips DNA
2) Elongation

- only 1 DNA strand is used as a template

3) Termination

- triggered by specific DNA sequences in the gene
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
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 tRNA, 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
Components needed to begin translation come together.
...INITIATION

2 On the assembled ribosome, a tRNA carrying the first amino acid is paired with the start codon on the mRNA. A tRNA carrying the second amino acid approaches.
ELONGATION...

3 The place on the ribosome where the first tRNA sits is called the P site. In the A site next to it, the second codon of the mRNA pairs with a tRNA carrying the second amino acid.
The first amino acid joins to the second by a peptide bond, and the first tRNA is released.
The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues.
...ELONGATION

6 The ribosome continues to move along the mRNA, and new amino acids are added to the polypeptide.
When the ribosome reaches a stop codon, the polypeptide is released.
Finally, the last tRNA is released, and the ribosome comes apart. The released polypeptide forms a new protein.
Summary of Translation

INITIATION

• ribosome assembles at specific AUG of mRNA

• ribosome binds 2 tRNA-AAs, 2 codons at a time
  • matching complementary anti-codons with mRNA codons

ELONGATION

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

• ribosome shifts 3 nucleotides (1 codon) on mRNA and repeats the process

TERMINATION

• “stop” codon causes translation to end
Table of the Genetic Code

If the DNA sequence is: CATGCCTGGGCAATAG
(transcription)

The mRNA copy is: CAUGCCUGGGCAAUAG
(translation)

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

*all proteins begin w/Met
Gene Expression in Prokaryotes

• gene expression is not necessarily "segregated"

• transcription & translation can occur simultaneously
Compartmentalization of Gene Expression in Prokaryotes

- as shown above, there is evidence of the segregation of transcription & translation in some prokaryotes
Gene Expression in Eukaryotes

1. One DNA strand serves as a template.
2. mRNA is processed before leaving the nucleus.
3. mRNA moves into cytoplasm and becomes associated with ribosomes.
4. tRNAs with anticodons carry amino acids to mRNA.
5. Anticodon-codon complementary base pairing occurs.
6. Peptide will be transferred to the tRNA-amino acid at the second binding site, and the tRNA at the first binding site will depart; ribosome then moves forward.
Splicing of Eukaryotic Transcripts

1. A gene composed of exons and introns is transcribed to RNA by RNA polymerase.

2. Processing involves ribozymes and proteins in the nucleus to remove the intron-derived RNA and splice together the exon-derived RNA into mRNA.

3. After further modification, the mature mRNA travels to the cytoplasm, where it directs protein synthesis.
2. Gene Regulation
Levels of Gene Regulation

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

**TRANSCRIPTION***
(regulation of rate at which gene is transcribed)

mRNA transcript stability
(“half-life” of transcripts)

*key level of regulation

**TRANSLATION**
(regulation of translation of mRNA)

post-translational modifications
(e.g., cleavage of polypeptides, addition of chemical groups)
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 (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 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”
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”
Summary of the *lac* operon

E. coli *lac* transcription-control genes

(a) - lactose
+ glucose
(low cAMP)

(b) + lactose
+ glucose
(low cAMP)

(c) + lactose
- glucose
(high cAMP)
3. DNA Replication & Mutation
DNA Replication

1. Enzymes unwind the parental double helix.

2. Proteins stabilize the unwound parental DNA.

3. The leading strand is synthesized continuously by DNA polymerase.

4. The lagging strand is synthesized discontinuously. RNA polymerase synthesizes a short RNA primer, which is then extended by DNA polymerase.

5. DNA polymerase digests RNA primer and replaces it with DNA.

6. DNA ligase joins the discontinuous fragments of the lagging strand.

Copyright © 2007 Pearson Education, Inc., publishing as Benjamin Cummings.
Features 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)
- **Primase** (makes RNA primers)
- **DNA Ligase** (“stitches” fragments together)
DNA Replication in Prokaryotes

- begins at the origin of replication (OriC)

- can only be completed if DNA is circular
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, DNA repair
- chemical mutagenesis
- high energy electromagnetic radiation
  - UV light, X-rays, gamma rays
Effects of Mutations

*insertions & deletions can cause “frame shifts”
4. Mechanisms of Gene Transfer
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

Harmless R strain

Heat-killed pathogenic cells

Mixed harmless and heat-killed pathogenic 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

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

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

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

D. 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.

1928!
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

(a) When an F factor (a plasmid) is transferred from a donor (F⁺) to a recipient (F⁻), the F⁻ cell is converted into an F⁺ cell.
Hfr Conjugation

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

- recipient can incorporate donor cell genes by recombination
- also useful for mapping bacterial genes based on the rate of transfer

**Hfr = “High frequency of recombination”**
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”

1. A phage infects the donor bacterial cell.
2. Phage DNA and proteins are made, and the bacterial chromosome is broken into pieces.
3. Occasionally during phage assembly, pieces of bacterial DNA are packaged in a phage capsid. Then the donor cell lyses and releases phage particles containing bacterial DNA.
4. A phage carrying bacterial DNA infects a new host cell, the recipient cell.
5. Recombination can occur, producing a recombinant cell with a genotype different from both the donor and recipient cells.
Key Terms for Chapter 8

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

Relevant Chapter Questions
rvw: 1-4, 8, 9, 11, 13   MC: 1, 2, 4, 5, 7-10