CHAPTER 8
MICROBIAL GENETICS

What is genetics?

- The science of heredity; includes the study of **genes**, how they **carry information**, how they are **replicated**, how they are **expressed**

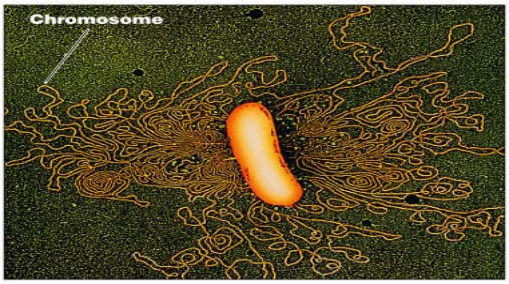
Terminology

- Genetics: Study of what genes are, how carry information, how information is expressed, and how genes are replicated
- Gene: Segment of DNA that encodes a functional product, usually a protein
- Genome: All of the genetic material in a cell
- Genomics:Molecular study of genomes
- Genotype: Genes of an organism
- Phenotype: Expression of the genes

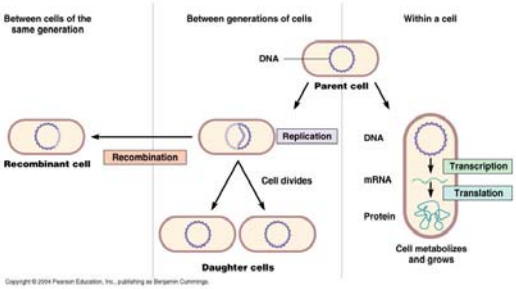
What do you know about DNA?

- Chromosomes made of DNA contain an organism's entire genome
- DNA codes for genes….genes code for proteins
- Chemical composition is nucleotides
- It exists in most cells as a double stranded structure

E. coli



Flow of information



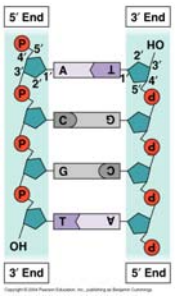
ENZYMES

- DNA gyrase: supercoils DNA (ciprofloxacin inhibits)
- Helicase: unwinds DNA
- DNA polymerase: adds nucleotides to make DNA (replication)
- RNA polymerase: adds nucleotides to make RNA from DNA (transcription)
- DNA ligase: joins Okazaki fragments

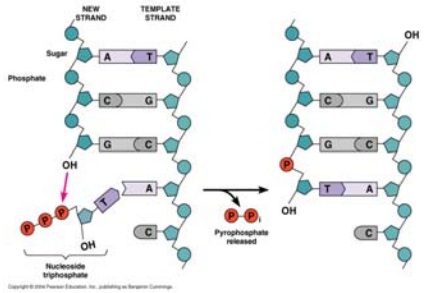
DNA

- Polymer of nucleotides: adenine, thymine, cytosine, guanine
- Double helix associated with proteins
- "Backbone" is deoxyribose-phosphate
- Strands held together by hydrogen bonds between AT and CG
- Strands are antiparallel

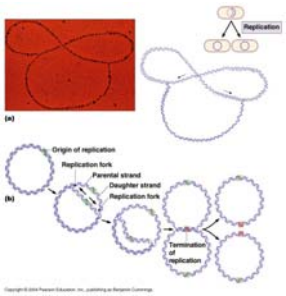
DNA strands are antiparallel



Nucleotides are added to the 3' position (OH group)



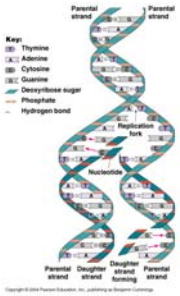
Bacterial DNA is circular



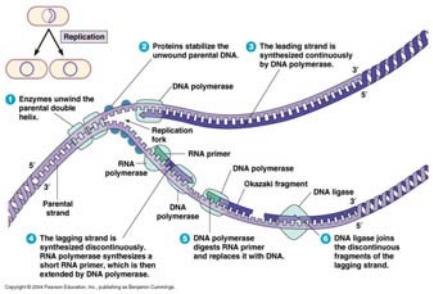
DNA Replication

- DNA is copied by DNA polymerase
  - In the 5′ → 3′ direction
  - Initiated by an RNA primer
  - Leading strand synthesized continuously
  - Lagging strand synthesized discontinuously
  - Okazaki fragments
  - RNA primers are removed and Okazaki fragments joined by a DNA polymerase and DNA ligase

DNA Replication



DNA replication…a closer look



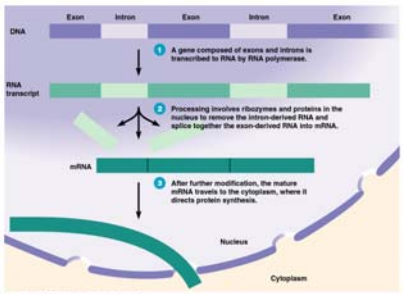
DNA Transcription to RNA

- DNA is transcribed to make RNA (mRNA, tRNA, and rRNA)
- RNA nucleotides: base pairing using DNA as template
- Transcription begins when RNA polymerase (enzyme) binds to the promotor sequence
- Transcription proceeds in the 5′ → 3′ direction
- Transcription stops when it reaches the terminator sequence

What are the possible products from transcription?

- Messenger RNA (mRNA)
- Transfer RNA (tRNA)
- Ribosomal RNA (rRNA)

Eukaryotic cells have RNA processing

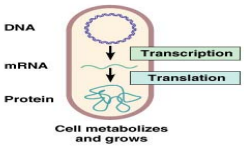


Translation: RNA to protein

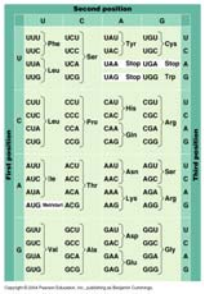
- What is needed for the process?
  - mRNA
  - Ribosomes
  - Amino acids
  - tRNA

Translation

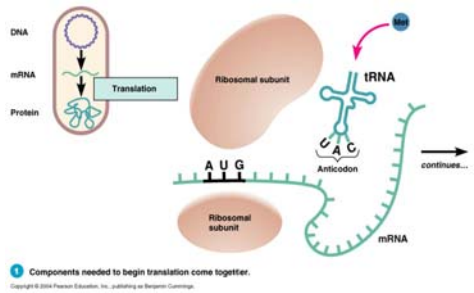
- mRNA is translated in codons (3 nucleotides)
- Translation of mRNA begins at the start codon: AUG
- Translation ends at a STOP codon: UAA, UAG, UGA



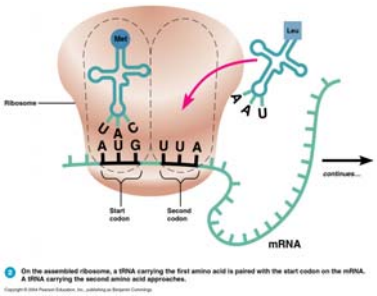
The Genetic code



Translation

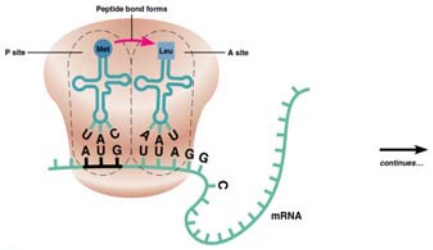


Translation



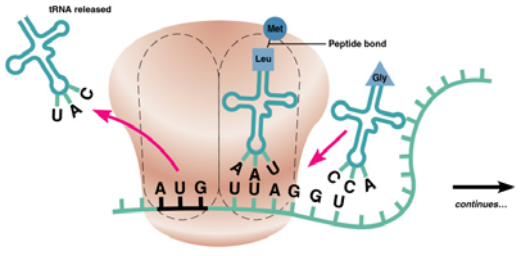
2 On the assembled ribosome, a tRNA carrying the first amino acid is paired with the start codon on the mRNA. A tRNA carrying the second amino acid approaches.

Translation



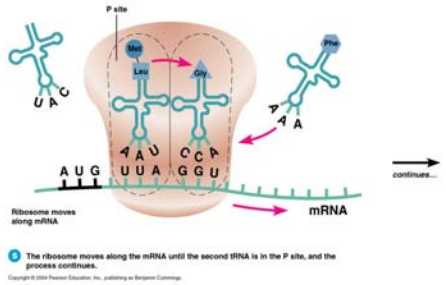
3 The place on the ribosome where the first tRNA sits is called the P site. In the A site next to it, the second codon of the mRNA pairs with a tRNA carrying the second amino acid.

Translation



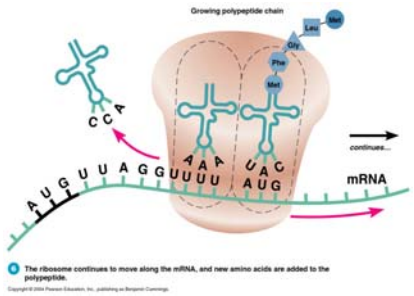
4 The first amino acid joins to the second by a peptide bond, and the first tRNA is released.

Translation



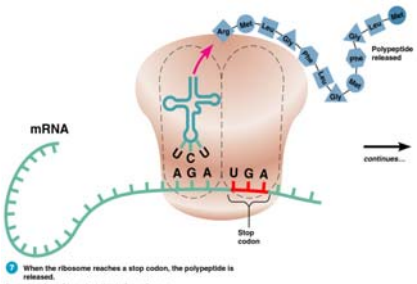
5 The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues.

Translation



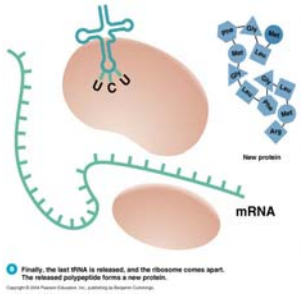
6 The ribosome continues to move along the mRNA, and new amino acids are added to the polypeptide.

Translation



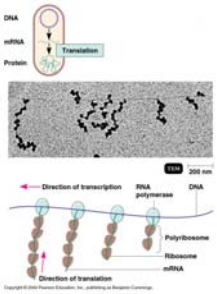
7 When the ribosome reaches a stop codon, the polypeptide is released.

Translation



Finally, the last tRNA is released, and the ribosome comes apart. The released polypeptide forms a new protein.

Both processes occur at the same time in bacteria…why??

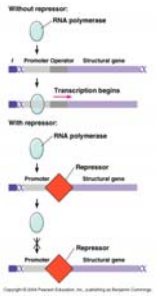


It is important to regulate protein synthesis

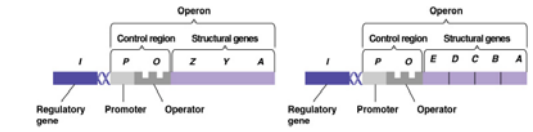
- Constitutive enzymes are expressed at a fixed rate
- Other enzymes are expressed only as needed
  - Repressible enzymes
  - Inducible enzymes

- Three types of protein regulation
  - Enyme inhibition (feedback inhibition)
  - Repression (tryptophan operon)
  - Induction (lactose operon)

Repression: regulation of gene expression

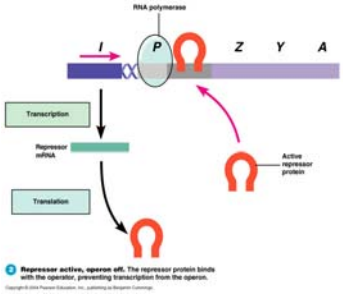


Operon: collection of closely associated genes

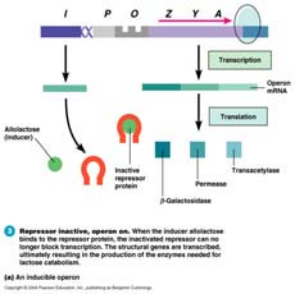


1 **Structure of the operon.** The operon consists of the promoter (*P*), and operator (*O*) sites, and structural genes which code for the protein. The operon is regulated by the product of the regulatory gene (*I*).

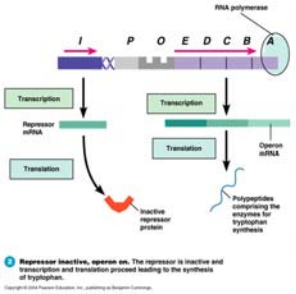
Lactose Operon: Inducible Enzyme



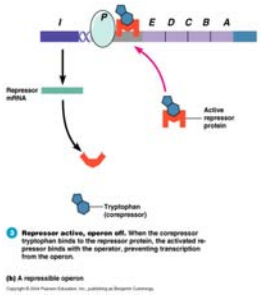
Lactose Operon



Tryptophan Operon: Repressible enzyme



Tryptophan Operon: Is tryptophan being synthesized?



What **conditions** are needed for the lactose operon to be turned "on"?

- No glucose
- Lactose present
- Increasing levels of cAMP
- cAMP binds to cAMP receptor protein, then lactose operon promoter
- RNA polymerase binds to promoter

If *E. coli* is growing in a flask with glucose and lactose…

What are mutations?

- Change in the base sequence of the DNA
- Do they always change the genetic code?

Causes of mutations in bacteria

- Most are spontaneous
- Errors made by DNA Polymerase
- UV light exposure
- Mutations may be neutral, beneficial, or harmful
- Mutagen: Agent that causes mutations
- Spontaneous mutations: Occur in the absence of a mutagen

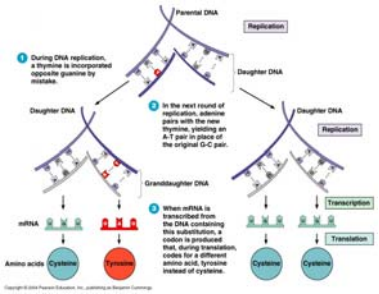
Types of Mutations

- Base-pair mutation
  - Missense mutation
  - Nonsense mutation
- Frameshift
  - Changes the reading frame

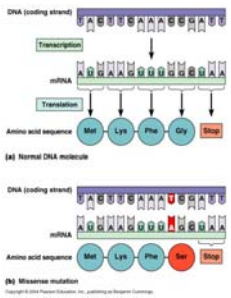
MUTATIONS

- **Base substitution (point mutation)**
  - Change in one base
- Missense mutation
  - Result in change in amino acid
- Nonsense mutation
  - Results in a nonsense codon

Base Pair Mutation



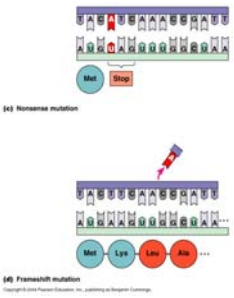
Missense Mutation



MUTATIONS

- **Frameshift mutation**
  - Insertion or deletion of one or more nucleotide pairs

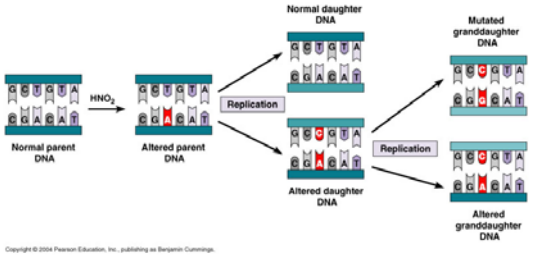
Frameshift Mutation



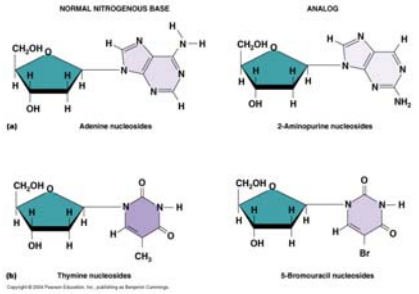
What can cause mutations?

- Chemicals (nitrous acid)
- Physical mutagens (uv light)
- Biological mutagens (transposons)

Nitrous acid as a chemical mutagen



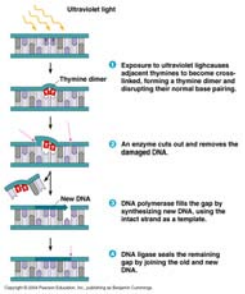
Nucleoside analogs are mutagens



PHYSICAL MUTAGENS

- Ionizing radiation (X rays and gamma rays) causes the formation of ions that can react with nucleotides and the deoxyribose-phosphate backbone.
- Nucleotide excision repairs mutations
- UV radiation causes thymine dimers
- Light-repair separates thymine dimers

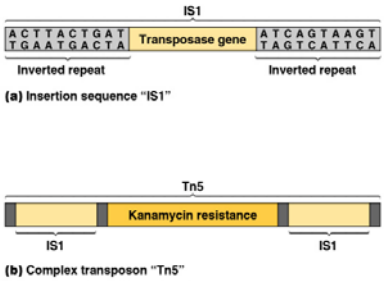
UV light as a mutagen



TRANSPOSONS

- Segments of DNA that can move from one region of DNA to another
- Contain insertion sequences for cutting and resealing DNA (transposase)
- Complex transposons carry other genes

TRANSPOSONS



FREQUENCY OF MUTATIONS

- Spontaneous mutation rate = 1 in 10^9 replicated base pairs or 1 in 10^6 replicated genes
- Mutagens increase to 10^{-5} or 10^{-3} per replicated gene

Why use bacteria to study mutations?

- Only have one chromosome…one copy of each gene
- Easy to grow

SELECTION OF MUTANTS

- Positive (direct) selection detects mutant cells because they grow or appear different.
- Negative (indirect) selection detects mutant cells because they do not grow.

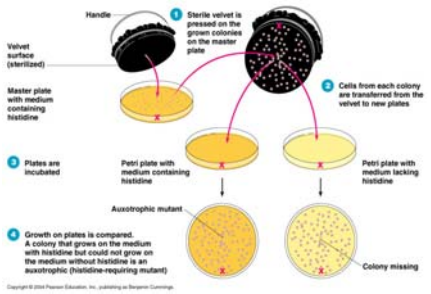
Direct selection

- Testing for traits that are easily identified
  - Colony color
  - Motility
  - Resistance to antibiotics

Indirect selection

- A way to look at traits that are not easily identified, at changes in metabolic pathways
- Replica plating
  - A way to identify AUXOTROPHS from PROTOTROPHS

Replica Plating: indirect selection



Testing chemicals for mutagenicity…Ames test