Bacterial Genetics

Microbiology
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Microbial Genetics

- Virtually all microbial traits are influenced by heredity.
- Inherited traits of microbes include—shape, structure features, metabolism, ability to move or behave in various ways, and ability to interact with other organisms (ex: causing disease)
- Individual organisms transmit these characteristics to offspring through genes
- Development of antibiotic resistance in microorganisms depends on genetics (ex: role of plasmids)
- Emerging diseases necessitate an understanding of bacterial genetics (ex: E. coli O157:H7)
- Studying bacterial genetics allows microbiologists to better understand relatedness among organisms and to better understand origins of potentially threatening organisms.

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History of DNA

TABLE 6.1 A Glimpse of DNA History: The Early Years

1869	Friedrich Miescher isolates nucleic acid from fish sperm and from pus cells obtained from discarded bandages.
1928	Griffith demonstrates first evidence of transfer of genetic material.
1943	Avery, MacLeod, and McCarty provide first evidence that DNA is the bearer of genetic information.
1952	Hershey and Chase demonstrate that phage carries DNA as genetic information.
1953	Double helix structure of DNA revealed by Watson, Crick, Franklin, and Wilkins.
1962	Nobel Prize awarded to Watson, Crick, and Wilkins for their discovery of the structure of DNA.

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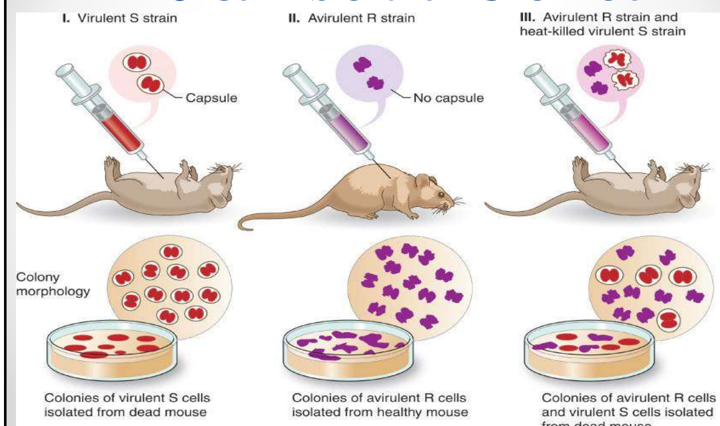
Griffith (1928)—genetic info can be transferred



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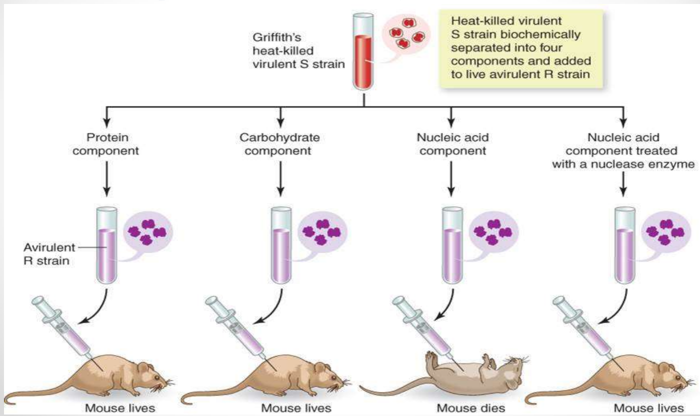
Avery, MacLeod & McCarthy (1943)—Transforming Principle; DNA carries the genetic information



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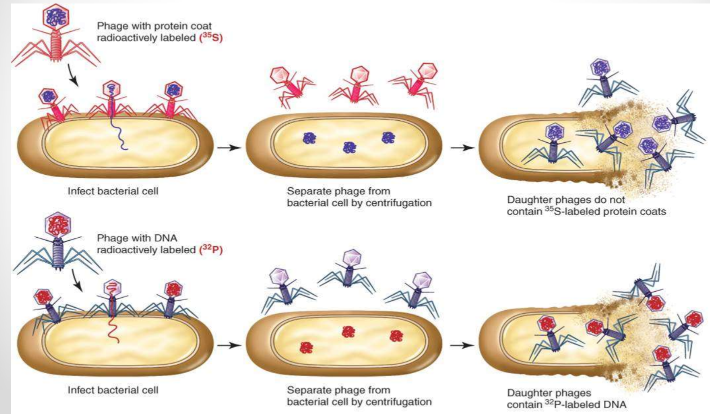
Hershey and Chase (1952)-

Phage carries DNA as genetic info; confirming DNA as genetic info component



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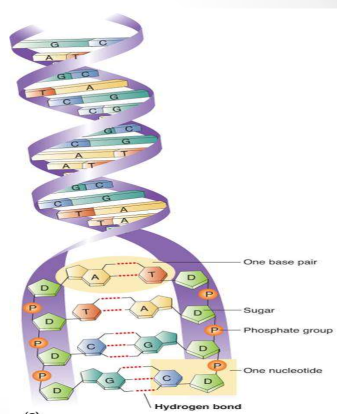
Watson & Crick—Proposed DNA Structure

This work identified the structure of DNA

Nobel prize was awarded to Watson, Crick, and Wilkins for their discovery of the structure of DNA



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Structure & Function of Genetic Material

Key terms:

Genetics = the science of heredity

Genome = the genetic information as organized within a cell (both chromosomal DNA and non-chromosomal)

Chromosomes = structures containing DNA that physically carry hereditary information; the chromosomes create the genes

Genes = segments of DNA that code for functional products

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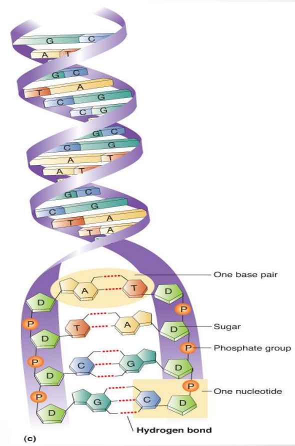
DNA Structure

DNA = deoxyribonucleic acid

- Consists of 2 chains of nucleotides
- Nucleotides consist of a base, a phosphate group, and a pentose sugar (deoxyribose)—next slide details this
- DNA within a cell exists as long strands of nucleotides twisted together in pairs to form a double helix



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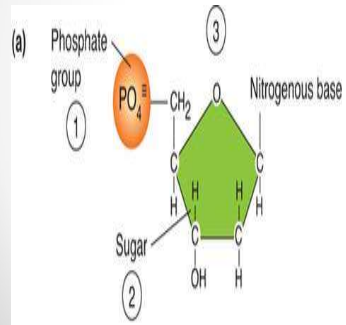
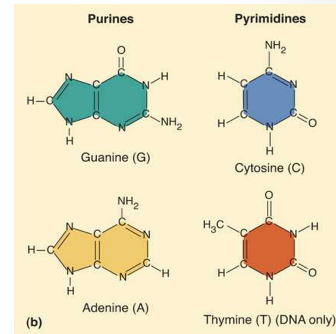
Nucleotide Structure

The Nucleotide



Nitrogenous Bases



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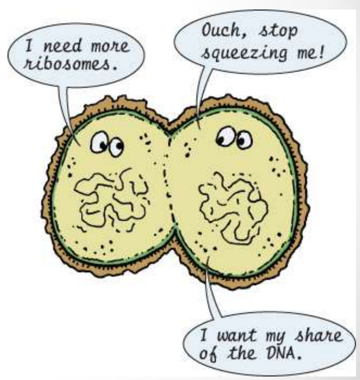
Bacterial Cell Division—via Binary Fission

Bacteria multiply via binary fission

Binary fission = an asexual mode of reproduction in which a cell splits into two new cells

Before this process can occur, DNA replication must occur



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DNA Replication

- In DNA replication, one "parental" double stranded DNA molecule is converted to two identical "daughter" molecules.
- Because the bases along the two strands of double-helical DNA are complementary, one strand can act as a template for the production of the other strand
- Semiconservative replication = the process of DNA replication in which each double-stranded DNA molecule contains one original strand and one new strand
- This allows for identical DNA material to be utilized in the process of binary fission.

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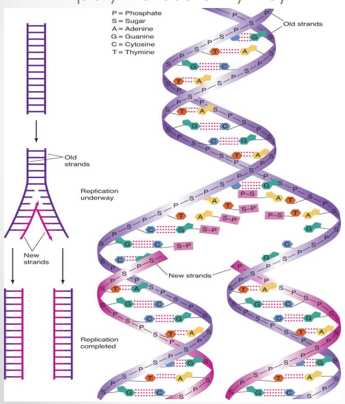
DNA replication

Hydrogen Bonds are enzymatically cleaved (DNA polymerase enzyme)



Chagraff's Rule of Complementary Base Pairs

- As the two strands unzip at specific sites, exposed bases on each strand are free to attach to free nucleotides
- Complementary base pairing occurs—A-T and G-C
- As unzipping proceeds, each original strand serves as a template for a new strand
- This process is typical of eukaryotic cells in which there are multiple linear pairs of chromosomes
- Let's look at the unique qualities of DNA replication for most prokaryotes (bacteria)

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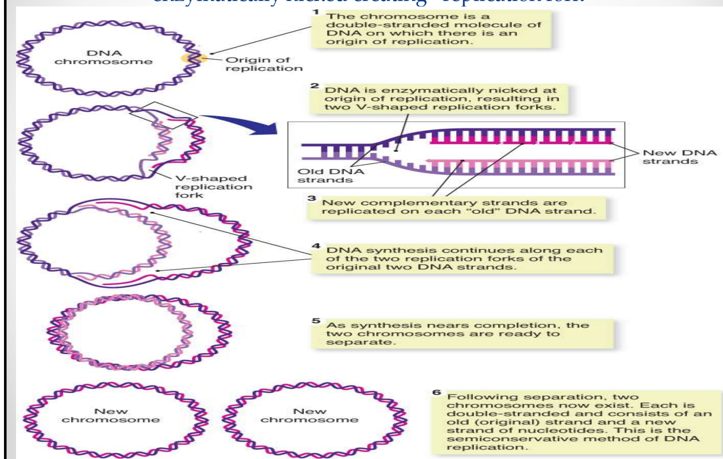
Loop Chromosome Structure in Bacterial Cells: chromosome is enzymatically nicked creating "replication fork"



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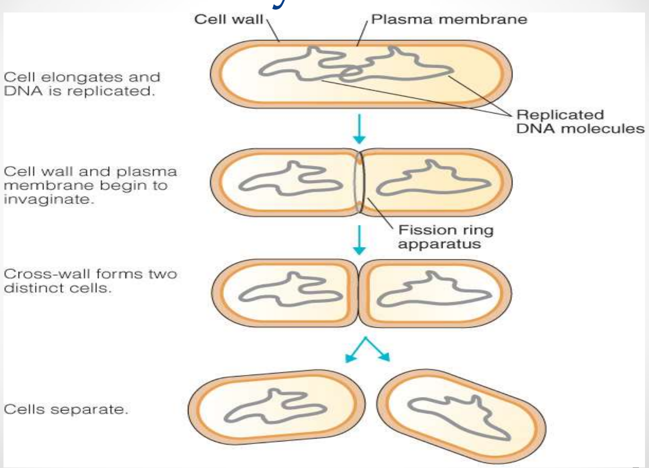
Binary Fission



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DNA—blueprint for the cell

- DNA carries the information needed to build proteins.
- Proteins are:
  - The most abundant compounds in the cell
  - Versatile
  - Key to genetic regulation for the cell
  - Major participants in cell's chemical reactions
  - Composed of amino acids linked together by peptide bonds (= polypeptides)

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Protein Synthesis

Involves 2 Sequential Processes:

1) Transcription

2) Translation

- Both steps involve DNA and RNA

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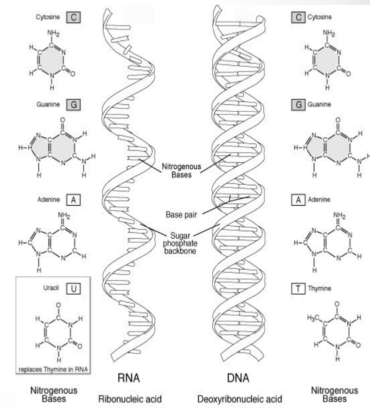
DNA vs. RNA

RNA is similar to DNA except:

- RNA is single-stranded
- Nucleotide uracil is substituted for thymine as the pair for adenine
- Ribose (pentose sugar) has one more oxygen in its structure than does deoxyribose



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RNA—Functional Classes

Messenger RNA: the type of RNA molecule that directs the incorporation of amino acids into proteins

Transfer RNA: the type of RNA molecule that brings amino acids to the ribosomal site where they are incorporated into proteins

Ribosomal RNA: they type of RNA molecule that is associated with ribosomes

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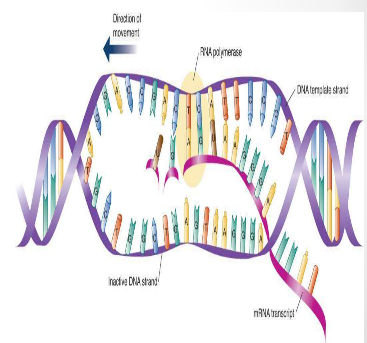
Stage 1: Transcription

- Genetic information is transferred from DNA to mRNA during this stage
- One strand of DNA serves as a template to make mRNA
- DNA is unzipped and the enzyme RNA polymerase is involved in making a complementary strand of mRNA
- Only a short segment of single-stranded DNA is transcribed at one time
- Transcription begins at a **promoter site** on the strand and ends at a **terminator sequence**
- Result: strand of mRNA complementary to original DNA blueprint has been formed
- Transcription takes place in the **nucleus** of the cell



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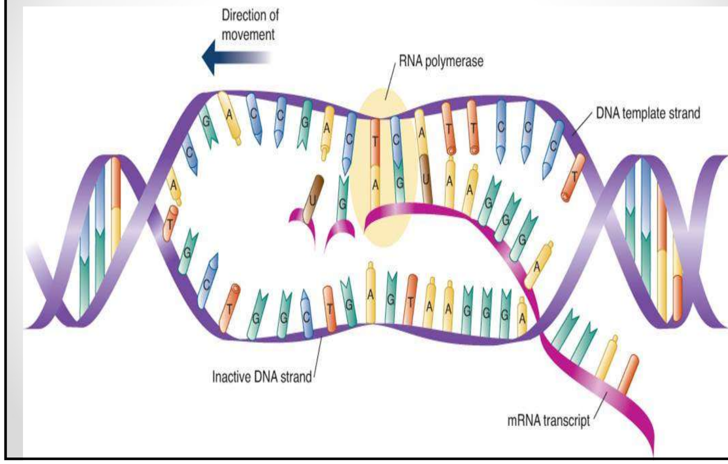
Transcription



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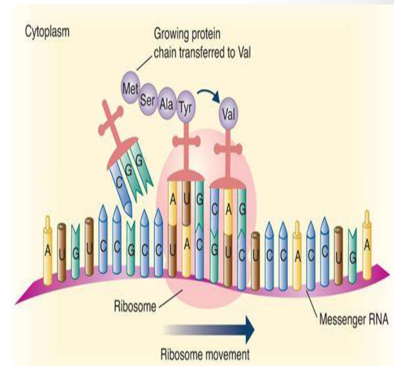
Stage 2: Translation

- The final stage of protein synthesis
- Takes place on ribosomes (protein synthesis organelles) in the **cytoplasm** of the cell
- mRNA moves from nucleus to cytoplasm

Translation = conversion of genetic info from mRNA into the language of proteins



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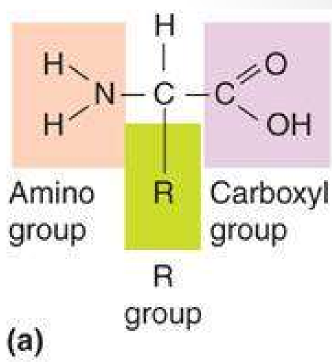
Amino Acids

= building blocks of protein

Consist of:

- Central carbon core
- Amino group—NH_2
- Carboxyl group—COOH
- Side chain (this is the portion of the amino acid that is unique and the interactions of these with other AA's give proteins their structure)—R group
- Amino acids are joined by peptide bonds to make proteins (polypeptide molecules)



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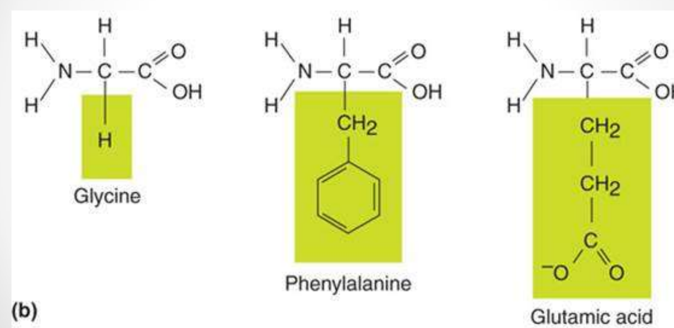
Side Chains—define the amino acid type



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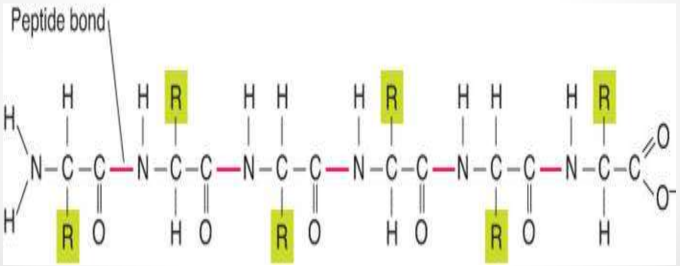
Peptide bonds—AA's build proteins



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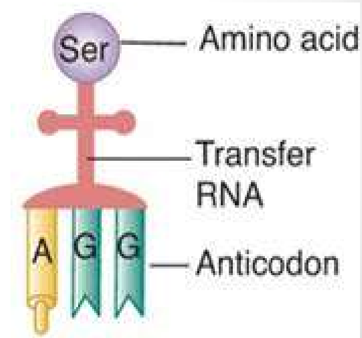
Transfer RNA (tRNA)

- Completion of translation process requires tRNA (transfer RNA)
- tRNA = molecules responsible for transferring a specific AA to the ribosome to build a polypeptide
- tRNA reads the mRNA nucleotides on the ribosome in groups of 3= **codons**
- Each codon related to a complementary **anticodon** on tRNA
- Each tRNA has a specific aticodon site to which an amino acid is chemically bonded



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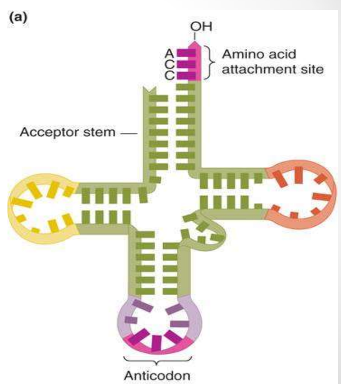
2 "business sites" on tRNA molecule

- Each tRNA molecule has an **anticodon site** which is chemically bonded to a specific amino acid.
- There are 2 business sites on each tRNA molecule.

1) Anticodon region (3 nucleotides which bind to mRNA codon)
2) Amino Acid binding site

- tRNA's carry the language and translate nucleic acid (mRNA) info into amino acid sequences (which make up the protein)=TRANSLATION



Schematic configuration

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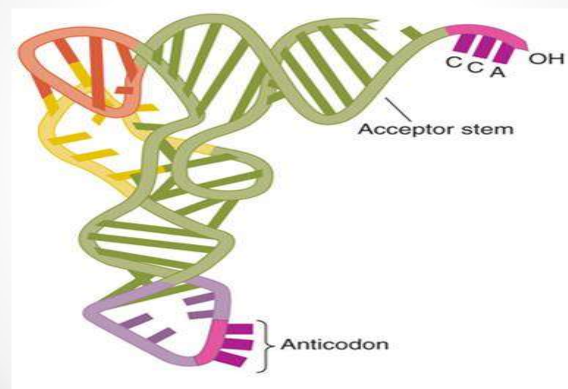
tRNA actually looks more like this…



Natural configuration

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Translation

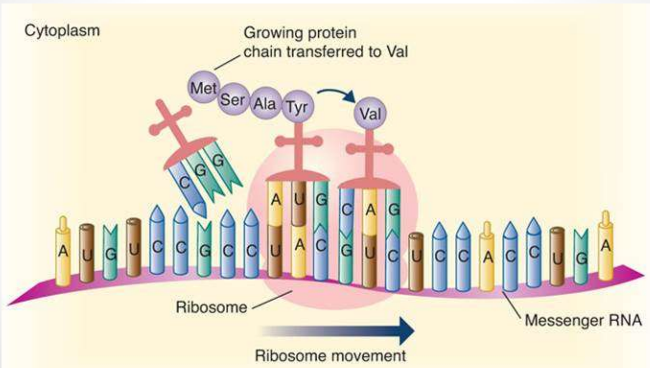

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TABLE 6.2 The Genetic Code Decoder

The genetic code embedded in mRNA is decoded by knowing which codon specifies which amino acid. On the far left column, find the first letter of the codon; then find the second letter from the top row; finally read up or down from the right-most column to find the third letter. In *Bacteria,* AUG codes for formylmethionine.

First base	Second base: U	Second base: C	Second base: A	Second base: G	Third base
U	UUU, UUC – Phe UUA, UUG – Leu	UCU, UCC, UCA, UCG – Ser	UAU, UAC – Tyr **UAA**, **UAG** – STOP	UGU, UGC – Cys **UGA** STOP UGG Trp	U C A G
C	CUU, CUC, CUA, CUG – Leu	CCU, CCC, CCA, CCG – Pro	CAU, CAC – His CAA, CAG – Gln	CGU, CGC, CGA, CGG – Arg	U C A G
A	AUU, AUC, AUA – Ile **AUG** Met (START)	ACU, ACC, ACA, ACG – Thr	AAU, AAC – Asn AAA, AAG – Lys	AGU, AGC – Ser AGA, AGG – Arg	U C A G
G	GUU, GUC, GUA, GUG – Val	GCU, GCC, GCA, GCG – Ala	GAU, GAC – Asp GAA, GAG – Glu	GGU, GGC, GGA, GGG – Gly	U C A G

Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartate; Cys = cysteine; Gln = glutamine; Glu = glutamic acid; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Trp = tryptophan; Tyr = tyrosine; Val = valine.

Codon Sequences for Amino Acids

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Gene Expression

- Both prokaryotes and eukaryotes have mechanisms for turning off or turning on gene expression
- Enzymes are major products of protein synthesis and are "biological catalysts"
- For the purpose of energy conservation, not all genes are turned on at all times

Constitutive enzymes: produced constantly as they are always in the "on" position for expression

Inducible enzymes: production is the result of genes that can be turned on or off depending on the circumstances

Operons: a group of functionally related genes that act as "on and off" switches

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Chromosomes—clarification

Chromosome—consists of long strands of DNA found or more succinctly, the structure into which DNA is organized

Chromosome number is constant for each species, no pattern or logic for chromosome numbers

Gene—piece or segment of that DNA

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TABLE 6.4 Chromosome Numbers[a]

Species	Chromosome Number
Typical prokaryote	1
Mosquito	6
Rye	14
Pigeon	18
Earthworm	36
Bread wheat	42
Human	46
Guppy	46
Gorilla	48
Plum tree	48
Potato	48
Cow	60
Chicken	78
Goldfish	94
Field horsetail	216
Adder's tongue fern	1,440

[a]Variation exists within species.

Table 06.T04: Chromsome Numbers

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TABLE 6.3 Bacterial Disease, Genomes, and Genes[a]

Bacterium	Disease	Genome (Base Pairs)	Number of Genes
Bordetella pertussis	Whooping cough (pertussis)	4,086,186	3,816
Borrelia burgdoferi	Lyme disease	910,724	850
Chlamydia trachomatis	Trachoma	1,044,459	911
Clostridium tetani	Tetanus	2,799,251	2,373
Escherichia coli	Enteritis	4,938,920	4,585
Helicobacter pylori	Ulcers	1,667,867	1,566
Listeria monocytogenes	Food poisoning	2,944,528	2,926
Mycobacterium tuberculosis	Tuberculosis	4,403,837	4,189
Yersinia pestis	Plague	4,702,289	4,167

[a]Variation exists within species depending upon strain.

Table 06.T03: Bacterial Disease, Genomes and Genes

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Bacterial Genetics

Mechanisms accounting for diversity in prokaryotic cells:

1) **Mutation**—random and haphazard; may produce genes adverse to survival
2) **Recombination**--more efficient; deals with existing genes
   - **Transformation**
   - **Transduction**
   - **Conjugation**

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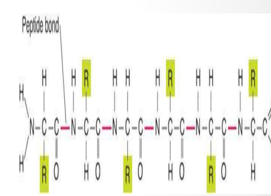
Mutations

- As previously discussed, the sequence of amino acids in a polypeptide determines the protein that results
- Even small mutations can lead to major functional changes for the cell
- **Point mutation**= one nucleotide is replaced with another nucleotide; insertion or deletion is taking place
- Can occur spontaneously OR can be the result of mutagens or transposons
- **Mutagens=** chemical or physical agents which can induce mutations
- **Transposons=** aka "jumping genes," genetic elements that move from one site on a chromosome to another or from a chromosome to a plasmid (or vice versa)



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Point Mutation—Sickle Cell Example

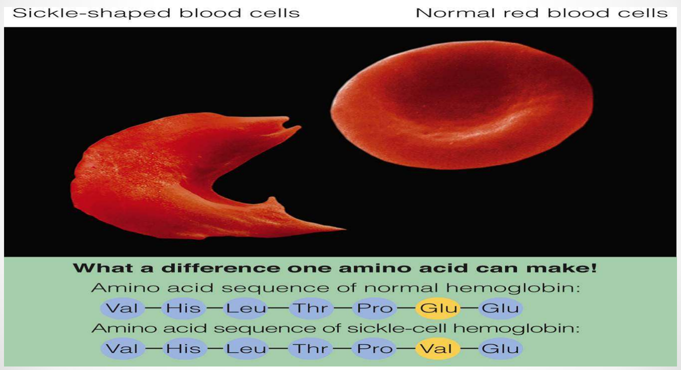

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TABLE 6.5 Mutations in Bacteria

Point mutation—a substitution of one nucleotide for another.

Nonsense mutation—one nucleotide change in a codon leading to a STOP codon; e.g., UAU and UAC code for amino acid tyrosine, but UAA and UAG are STOP codons.

Missense mutation—one nucleotide changes in a codon leading to a different amino acid, e.g., GUU codes for amino acid valine and GCU codes for amino acid alanine.

Insertion—addition of one nucleotide leads to a change in the reading frame of codons; e.g., the nucleotide sequence AGU CCA UUU ACC codes for the amino acid sequence of serine, proline, phenylalanine, and threonine. The addition of G in the second codon in front of A establishes the sequence AGU CCG AUU UAC, which codes for the amino acids serine, proline, isoleucine, and tyrosine.

Deletion—deletion of one nucleotide leads to a change in the reading frame (a frameshift mutation); for example, the nucleotide sequence AGU CCA UUU ACG codes for the amino acids serine, proline, phenylalanine, and threonine. However, if the nucleotide A in the second codon is deleted, the sequence is now AGU CCU UUA CG, resulting in the amino acids serine, proline, and leucine (a frameshift mutation).

Mutagens—physical or chemical agents introduced to cause mutation.

Physical—ionizing radiation (X-rays and gamma rays), nonionizing ultraviolet light.

Chemical—nucleotide analogs (similar to nucleotides).

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Recombination

3 processes for recombination of bacterial genes: transformation, transduction, and conjugation

- Although these processes are distinct, the following factors are common to each process:
  - All are unidirectional
  - Multiplication is not an outcome
  - All are examples of **horizontal transfer** (vs. vertical)
  - All are based on **homologous recombination**
  - All occur in nature, although described as laboratory phenomena
  - All require integration of foreign donor DNA into host DNA
  - All result in new gene combinations

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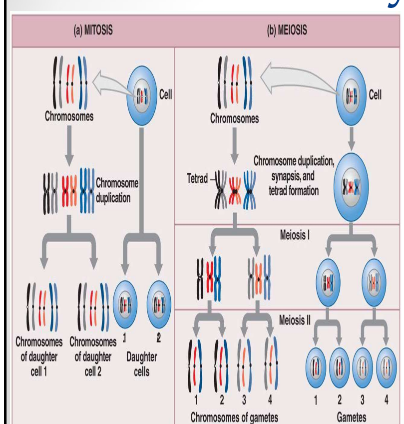
Recombination—in Eukaryotes



Eukaryotes:

- Recomination in sexually reproducing organisms is **vertical**
- **Vertical recombination—** involves the fusion of male & female gametes
- Gametes= haploid—carrying ½ of the genetic info
- Process is meiosis
- "genetic gamble" = new combination; reproduction

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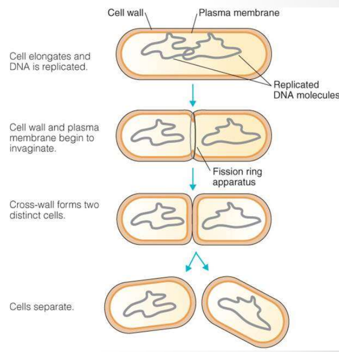
Recombination—in Prokaryotes

- **Strategy for recombination in prokaryotes is separate and distinct from reproduction**
- Remember—bacteria reproduce by binary fission in which two identical daughter cells are produced asexually →



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Recombination Types

TABLE 6.6 Summary and Comparison of Transformation, Transduction, and Conjugation

Type	Characteristics	Discovery
Transformation	Uptake of naked DNA Recipient cells need to be competent	Griffith, 1928
Transduction	DNA carried by bacteriophage from donor to recipient Generalized transduction result of DNA fragments in phage following lysis of bacterial host cell Specialized transduction result of faulty breakout of prophage in the lysogenized host cell	Avery, Macleod, McCarty, 1944
Conjugation	Contact between donor and recipient via F pilus F^+ to F^- Hfr to F^-	Lederberg, Tatum, 1946

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Transformation

= the process in which genes are transferred from one bacterium to another as "naked" fragments of DNA into competent cells

→ Process requires competence and homology for transfer to occur; mediated by enzymes
→ Naked DNA is integrated into the host DNA

Competence: cells have the ability to take up DNA from the environment resulting in gene transformation; most cells are not naturally competent—can be altered in lab settings
→ Competence results from alterations in the cell wall that make it permeable to large DNA molecules

Homology: refers to stretches of DNA with identical or closely related sequences

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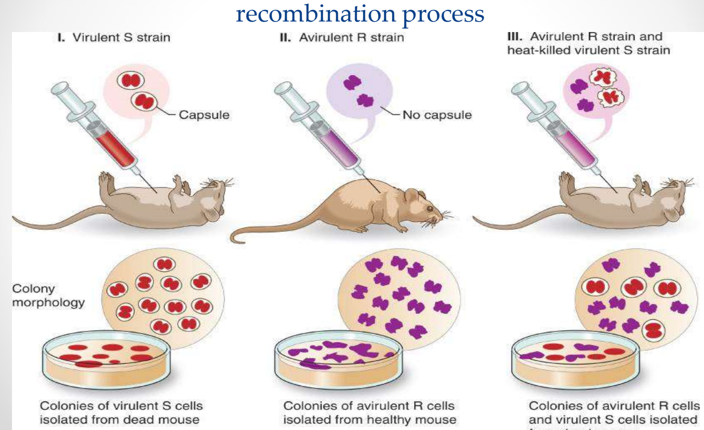
Transformation—Griffith's experiment exemplifies this recombination process



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Transduction—2 types

= the transfer of DNA from one cell to another by a bacteriophage

Generalized transduction = the transfer of bacterial chromosome fragments from one cell to another by a bacteriophage (lytic process)

Specialized transduction = process of transferring a piece of cell DNA adjacent to a prophage to another cell

- Characteristic of temperate (lysogenic) phages
- Viral nucleic acid is incorporated into bacterial chromosome by homologous recombination
- Incorporated phage DNA or piece of it = prophage

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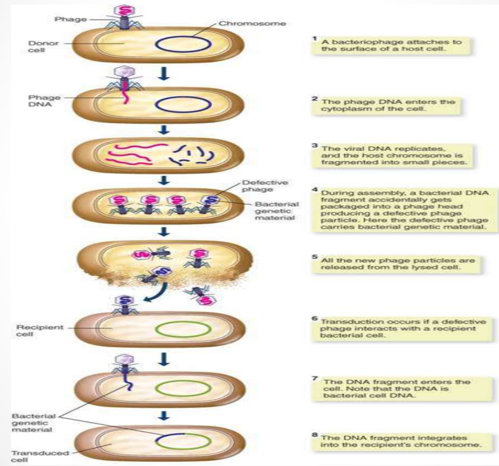
Generalized Transduction



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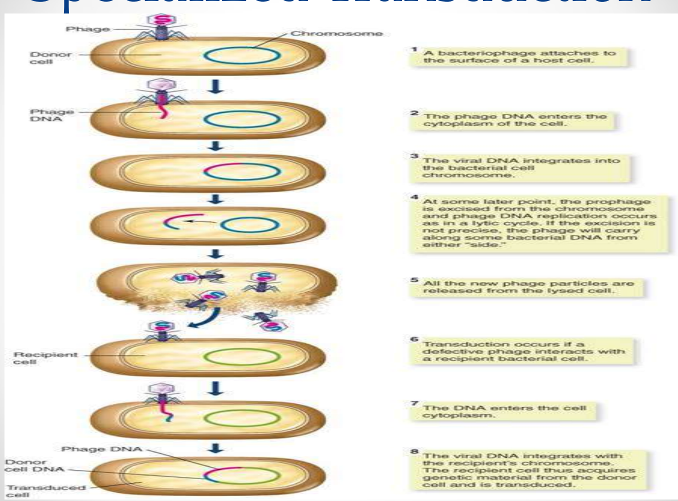
Specialized Transduction



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Conjugation

= the transfer of genetic material from once cell to another involving cell-to-cell contact

- Looks similar to mating, but doesn't meet the criteria for sexual reproduction (no gametes)
- Conjugation is mediated by plasmids.
- Plasmids differ from bacterial chromosomes in that the genes they carry are usually not essential for the growth of the cell under normal conditions.
- The plasmids responsible for conjugation are transmissible between cells during conjugation
- Requires that cells involved are of opposite mating type (ex: donor cells must carry plasmid while recipient cell does not)

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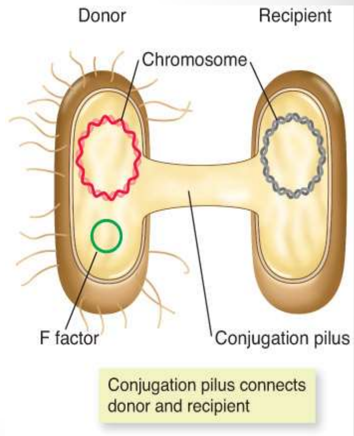
Conjugation

Plasmid—referred to in conjugating cells as "F Factor" = fertility factor

Donor cell is F+ = has the F factor plasmid

Recipient cell is F- = no plasmid with fertility factor

Some F plasmids produce F pili (sex pili or conjugation pili) which act as a bridge between donor and recipient cells



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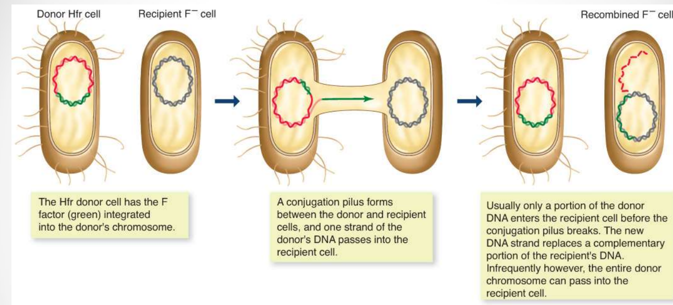

Figure 06.F17: Bacterial conjugation: Hfr to F –. (The pilus may not act as a channel as shown.)

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Transfer of Genetic Info without Plasmids

- Transfer of genetic material is not limited to plasmid containing cells.
- F factor may be integrated into the chromosome of some cells.
- HFr (high frequency recombination) = cell type in which frequent recombination occurs
- The integration of the plasmid into the chromosome is reversible, which leads to a mixed population of Hfr and F+ cells

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Why We Care About Bacterial Genetics…

- All mechanisms of genetic exchange in bacteria—transformation, transduction, conjugation, and mutation—are responsible for bacterial diversity
- Diversity leads to the ability of bacteria to adapt to new environments—survival of the fittest at the genetic and molecular levels
- The "fittest" are those bacteria in a diverse population with genes that allow them to adapt, survive and reproduce under hostile circumstances
- Much of the antibiotic resistance that we are currently encountering is the result of genetic exchange/diversity of these organisms.

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