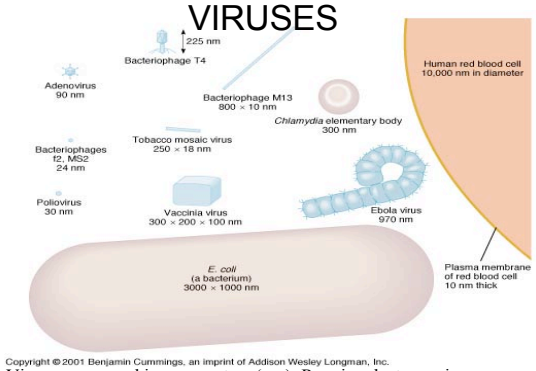
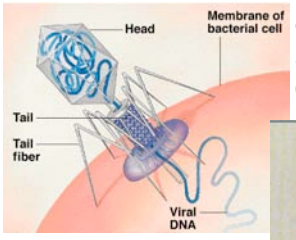
## VIRUSES

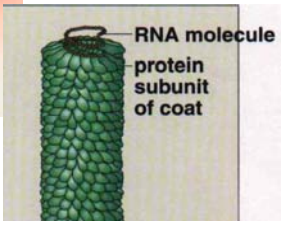



Viruses measured in nanometers (nm). Require electron microscopy.

## Viruses are small packages of genes




**Consist of protein coat around nucleic acids (DNA or RNA)**




## Obligate intracellular parasites

- Cannot grow or reproduce by itself
  - Have no independent metabolic pathways for anabolic synthesis
- Reproduce (replicate) only by using host cell machinery
- Non-cellular
- (Is it alive?)

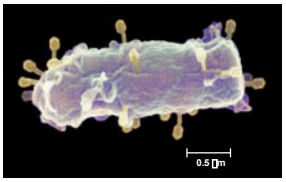
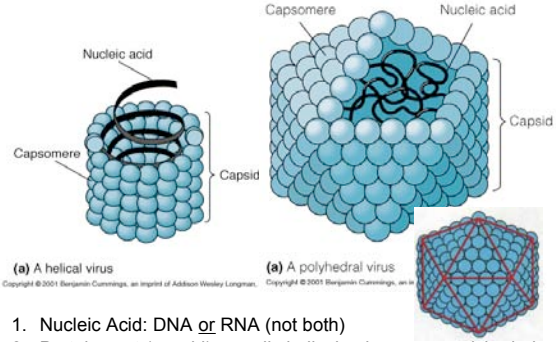



Figure 18.1

## Basic virus structure



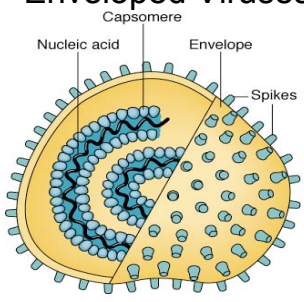

1. Nucleic Acid: DNA or RNA (not both)
2. Protein coat (capsid): usually helical-columnar or polyhedral.

## Classification of Viruses

Based upon

- Morphology of **capsid**
- Presence of an **envelope** around the capsid
- Type of nucleic acid
  - **DNA or RNA**
  - **Double-stranded (ds) or single-stranded (ss)**
  - **Circular or linear**
  - (Most are dsDNA [in bacteria] or ssRNA [in plants & animals])

## Enveloped Viruses




**(a)** An enveloped helical virus

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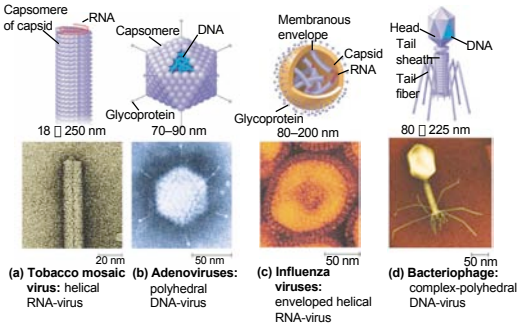
Envelope derived from host cell membrane + viral protein spikes.

## Classification of Viruses




**(a) Tobacco mosaic virus:** helical RNA-virus

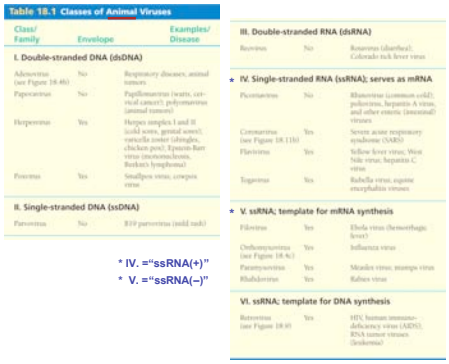
**(b) Adenoviruses:** polyhedral DNA-virus

**(c) Influenza viruses:** enveloped helical RNA-virus

**(d) Bacteriophage:** complex-polyhedral DNA-virus

Figure 18.4. Colorized TEMs

## Classification of Viruses

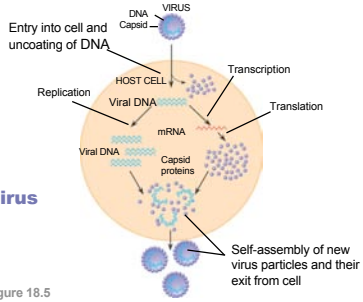



* IV. ="ssRNA(+)"

* V. ="ssRNA(–)"

## General viral reproductive cycle

- Viruses use enzymes, ribosomes, nucleotides, amino acids, etc. *of host cells* to synthesize progeny viruses




**a DNA virus**

Figure 18.5

## The Lytic Cycle (virulent phage)

- **Bacteriophage T2 or T4**

**1 Attachment.** The T4 phage uses its tail fibers to bind to specific receptor sites on the outer surface of an *E. coli* cell.

**2 Entry of phage DNA and degradation of host DNA.** The sheath of the tail contracts, injecting the phage DNA into the cell and leaving an empty capsid outside. The cell's DNA is hydrolyzed.

**5 Release.** The phage directs production of an enzyme that damages the bacterial cell wall, allowing fluid to enter. The cell swells and finally bursts, releasing 100 to 200 phage particles.

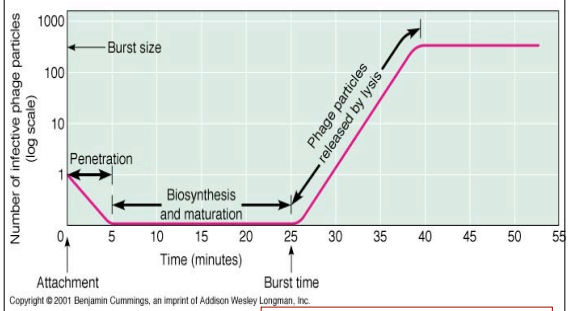
Phage assembly


**4 Assembly.** Three separate sets of proteins self-assemble to form phage heads, tails, and tail fibers. The phage genome is packaged inside the capsid as the head forms.

**3 Synthesis of viral genomes and proteins.** The phage DNA directs production of phage proteins and copies of the phage genome by host enzymes, using components within the cell.

Figure 18.6

## One-step Growth Curve



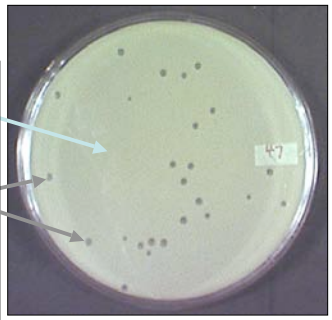



Attachment to lysis in 25 minutes!

## The Lytic Cycle

- **Bacteriophage**

- Agar plate with "bacterial lawn" (solid white field of bacteria)
- Plaques: clear, bacteria-free region growing around one original viral-infected bacterium



## Lytic / Lysogenic Cycles (temperate phage)

- **Bacteriophage**

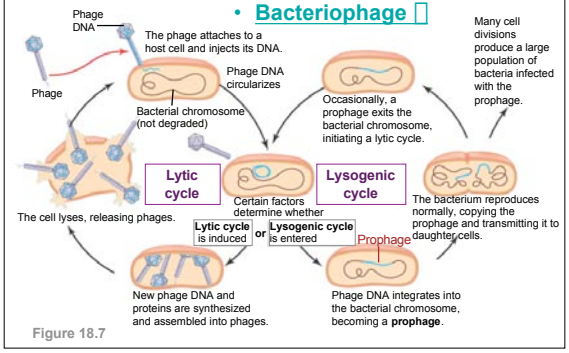



Figure 18.7

## Lytic Cycles and the Transduction of Bacterial Hosts

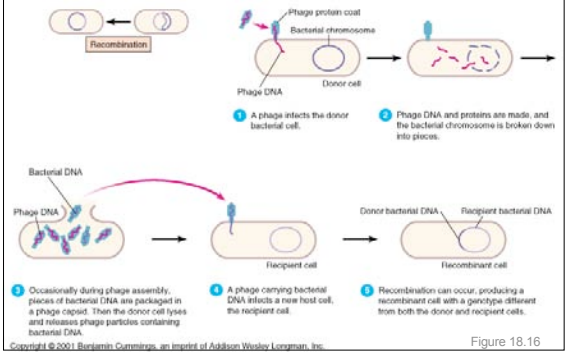
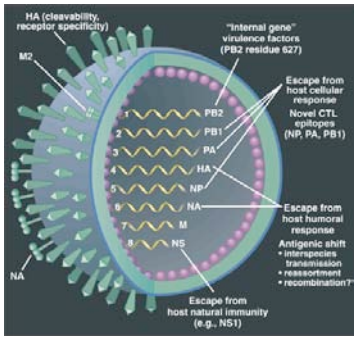



Figure 18.16

## Enveloped RNA-virus structure

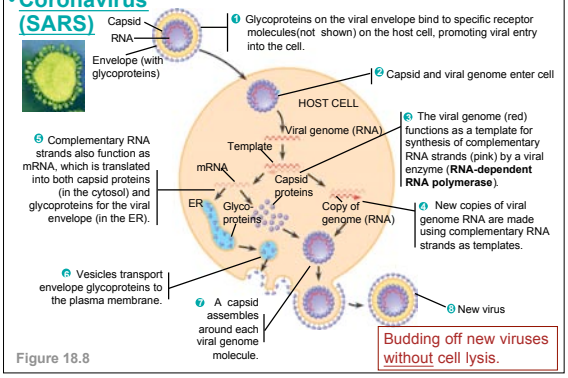
- **Influenza virus**




- **Envelope membrane**
- **8 RNA segments**
- **10 proteins**

## Enveloped ssRNA(–) virus cycle

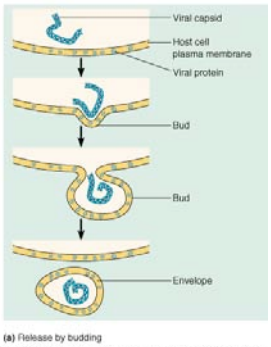
- **Coronavirus (SARS)**




Budding off new viruses without cell lysis.

Figure 18.8

## Enveloped RNA-virus cycle






Budding off new viruses without cell lysis.

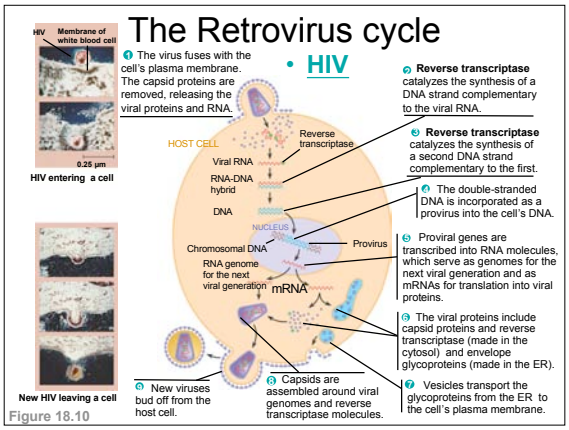
## RNA-virus cycles

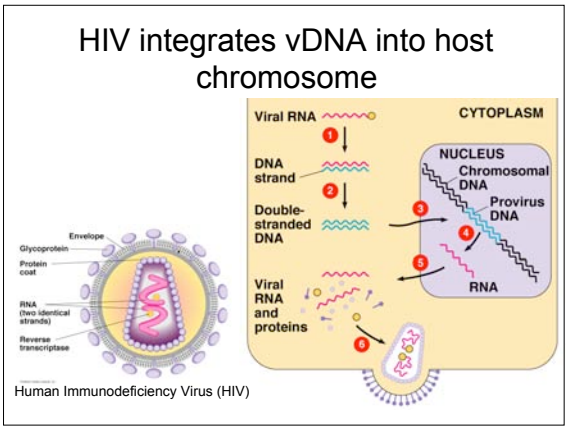
**ssRNA (+) viruses**

- The (+) strand acts as an mRNA
  - Can be translated by host cell ribosomes to make viral proteins
- Including **RNA-dependent RNA polymerase** (RDRP)
  - Uses RNA as a template to replicate more RNA
- RDRP replicates (+) strand to produce complementary (–) strands, and then the (–) strands to make more (+) strands
- ↑↑ (+) strands  more viral protein & genome for newly assembled viruses
- E.g., poliovirus & cold virus

**ssRNA (–) viruses**

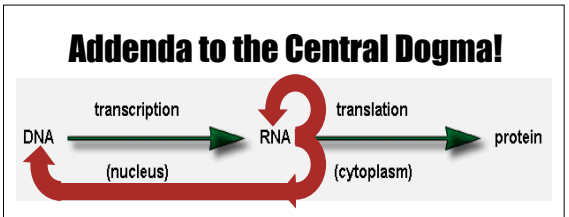
- The (–) strand cannot act as an mRNA
  - Cannot be translated
- The virus must provide some RNA-dependent RNA polymerase (RDRP)
- RDRP replicates (–) strand to produce (+) strands
- the (+) strands are templates to translate viral proteins and for RDRP to replicate (–) strands
- Genomic (–) strands & viral proteins assembled into new viruses
- E.g., rabies virus & influenza virus




## Viroids

- Small loop of RNA with no capsid.
  - Infect plant cells.



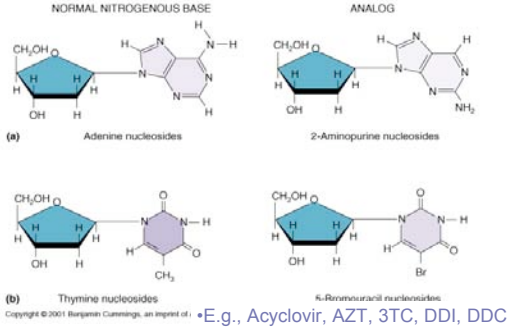

- 1950s: All known life forms use the same mechanism to produce protein
- 1970s: RNA viruses use RNA to make RNA!
- 1990s: Retroviruses use RNA to make DNA!!

## Viral-caused pathology

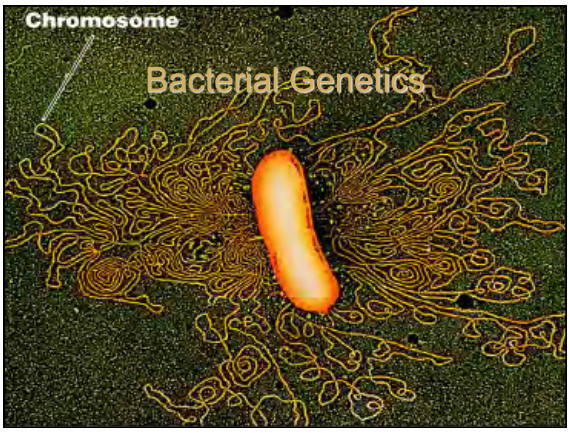
- Cell lysis & death
- Release of intracellular degradation enzymes into surrounding tissues.
- Toxic viral proteins
- Disruption of cellular function
  - Diminished vital functions
  - Production of toxic metabolites

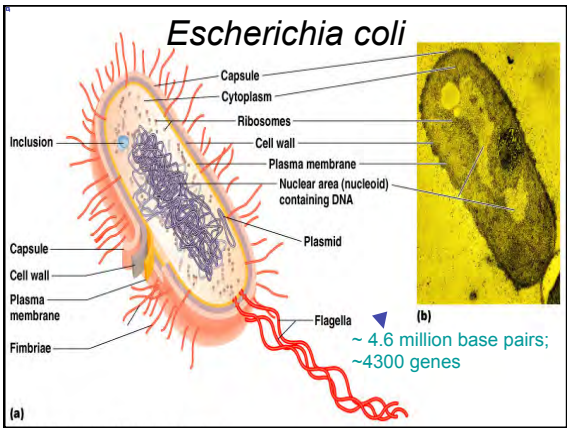
## Nucleoside Analogs

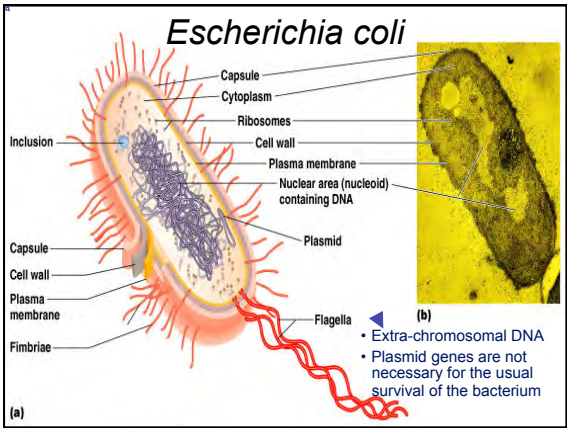
as therapeutic inhibitors of viral polymerases

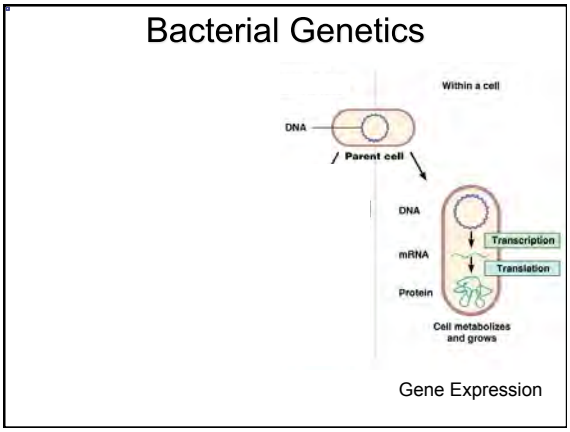


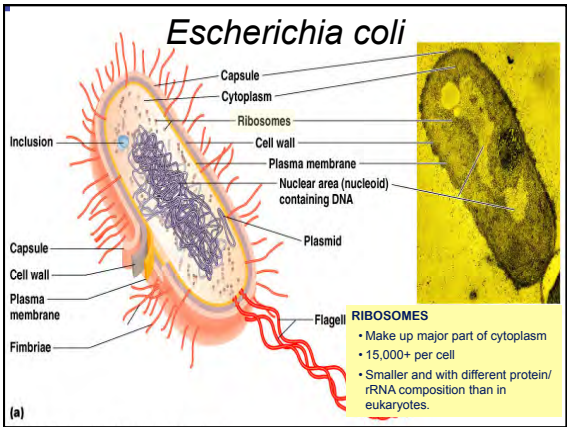

- E.g., Acyclovir, AZT, 3TC, DDI, DDC

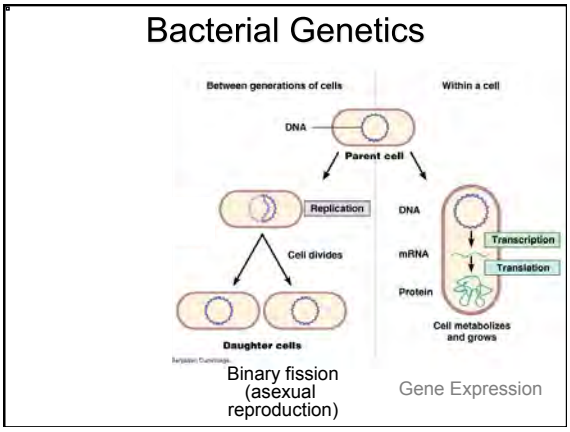

Chromosome
Bacterial Genetics


Escherichia coli
Capsule
Cytoplasm
Ribosomes
Cell wall
Plasma membrane
Nuclear area (nucleoid) containing DNA
Plasmid
Inclusion
Capsule
Cell wall
Plasma membrane
Fimbriae
Flagella
(a)
(b)
~ 4.6 million base pairs; ~4300 genes


Escherichia coli
Capsule
Cytoplasm
Ribosomes
Cell wall
Plasma membrane
Nuclear area (nucleoid) containing DNA
Plasmid
Inclusion
Capsule
Cell wall
Plasma membrane
Fimbriae
Flagella
(a)
(b)
• Extra-chromosomal DNA
• Plasmid genes are not necessary for the usual survival of the bacterium


Bacterial Genetics
Within a cell
DNA
Parent cell
DNA
mRNA
Protein
Transcription
Translation
Cell metabolizes and grows
Gene Expression


Escherichia coli
Capsule
Cytoplasm
Ribosomes
Cell wall
Plasma membrane
Nuclear area (nucleoid) containing DNA
Plasmid
Inclusion
Capsule
Cell wall
Plasma membrane
Fimbriae
Flagell
(a)
RIBOSOMES
• Make up major part of cytoplasm
• 15,000+ per cell
• Smaller and with different protein/ rRNA composition than in eukaryotes.


Bacterial Genetics
Between generations of cells
Within a cell
DNA
Parent cell
Replication
Cell divides
Daughter cells
DNA
mRNA
Protein
Transcription
Translation
Cell metabolizes and grows
Binary fission (asexual reproduction)
Gene Expression

## Replication of the circular bacterial chromosome or plasmid

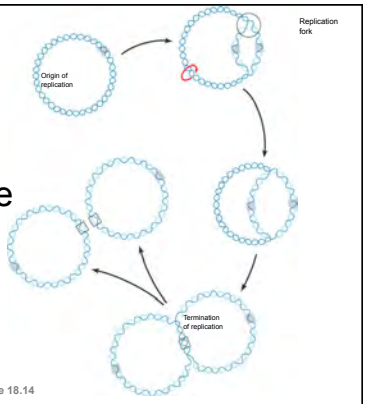
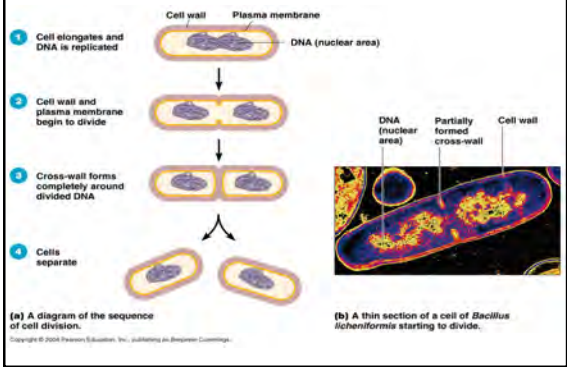


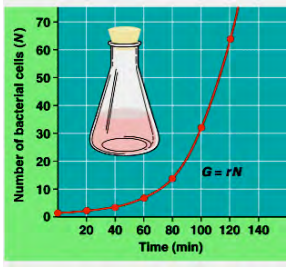

Figure 18.14

## Binary Fission



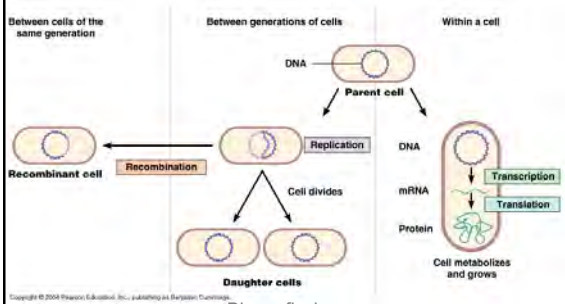

## Exponential Growth

| Time | Number of Cells | |
|---|---|---|
| 0 minutes | 1 | $= 2^0$ |
| 20 | 2 | $= 2^1$ |
| 40 | 4 | $= 2^2$ |
| 60 | 8 | $= 2^3$ |
| 80 | 16 | $= 2^4$ |
| 100 | 32 | $= 2^5$ |
| 120 (= 2 hours) | 64 | $= 2^6$ |
| 3 hours | 512 | $= 2^9$ |
| 4 hours | 4096 | $= 2^{12}$ |
| 8 hours | 16,777,216 | $= 2^{24}$ |
| 12 hours | 68,719,476,736 | $= 2^{36}$ |




- Population multiplies by a constant factor when growth rate not limited by resources.
- *In vitro* doubling time = 20 minutes
- *In situ* doubling time = 12 hours

## Bacterial Genetics




Recombinant reproduction | Binary fission (asexual reproduction) | Gene Expression

## HOW BACTERIA GAIN NEW GENETIC INFORMATION

- MUTATIONS
- RECOMBINATION
  - TRANSDUCTION
  - TRANSFORMATION
  - CONJUGATION
- TRANSPOSITION

## Mutation Rates

- # of mutations per cell generation
  - Higher in bacteria than in animal cells
    - Lack level of repair mechanisms
  - Yet only 1 mutation out of $10^7$ to $10^8$ replicated genes
- But most cell cultures contain about $10^9$ cells per ml
  - and the average bacterial chromosome is about 4000 genes

∴ Each ml contains ~40,000 mutations that were not there one generation before

∴ Each ml contains ~10 cells with a mutation in any specific gene

HOW BACTERIA GAIN NEW GENETIC INFORMATION

## Mutations vs. Recombination

**EXPERIMENT** Researchers had two mutant strains, one that could make arginine but not tryptophan (*arg*$^+$ *trp*$^-$) and one that could make tryptophan but not arginine (*arg*$^-$ *trp*$^+$). Each mutant strain and a mixture of both strains were grown in a liquid medium containing all the required amino acids. Samples from each liquid culture were spread on plates containing a solution of glucose and inorganic salts (minimal medium), solidified with agar. Only bacteria that can make both amino acids should survive on this minimal medium.

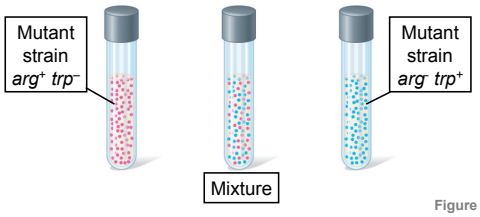



Figure 18.15

HOW BACTERIA GAIN NEW GENETIC INFORMATION

## Mutations vs. Recombination

**RESULTS** Only the samples from the mixed culture, contained cells that gave rise to colonies on minimal medium, which lacks amino acids.

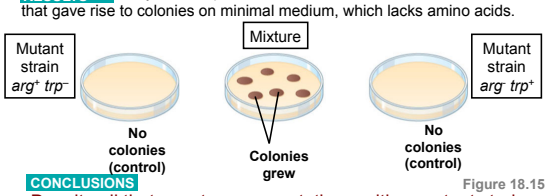



Figure 18.15

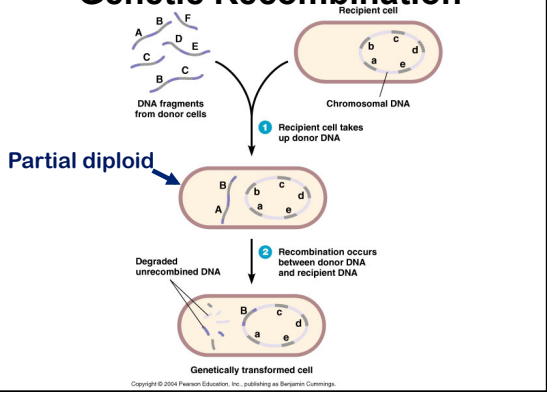
**CONCLUSIONS**

1. Despite all that spontaneous mutation, neither mutant strain re-acquired the ability to synthesize amino acids.
2. Thus, each cell from the mixture that formed a colony on the minimal medium must have acquired one or more genes from a cell of the other strain by **genetic recombination**.

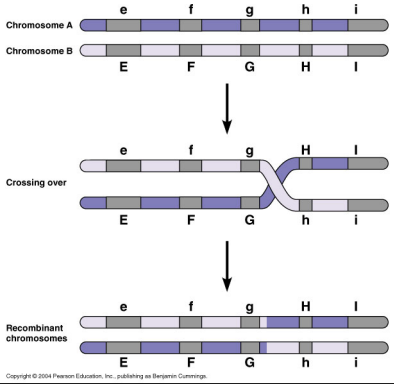
## HOW BACTERIA GAIN NEW GENETIC INFORMATION

- MUTATIONS
- **RECOMBINATION**
  - TRANSDUCTION
  - TRANSFORMATION
  - CONJUGATION
- TRANSPOSITION

## Genetic Recombination

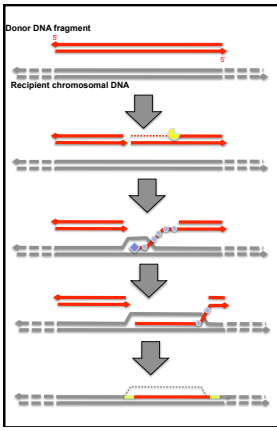



## Genetic Recombination




**Crossing over**

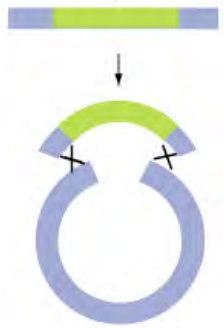
- Exchange of genes between two DNA molecules




## Bacterial Recombination

- Donor DNA is **nicked** and digested to form single strand end
- **Strand invasion**: Recombination protein complex
  - binds donor ssDNA to recipient dsDNA
  - unzips recipient dsDNA
  - complementary recipient strand is displaced [D-loop]
  - **homologous** donor and recipient DNA complementary pair
- **Branch migration**: additional donor DNA displaces more D-loop
- **Termination**:
  - D-loop excised or degraded
  - DNA-polymerase fills in gaps
  - Ligase joins ends

## Bacterial Recombination



**Non-homologous DNA** may be recombined if it is flanked by homologous regions of DNA

## HOW BACTERIA GAIN NEW GENETIC INFORMATION

- MUTATIONS
- RECOMBINATION
  - **TRANSDUCTION**
  - **TRANSFORMATION**
  - **CONJUGATION**
- TRANSPOSITION

## TRANSDUCTION
— GENERALIZED

1. Phage infects bacterial cell that has alleles $A^+$ and $B^+$
2. Host DNA (brown) is fragmented, and phage DNA and proteins are made. This is the donor cell.
3. A bacterial DNA fragment (in this case a fragment with the $A^+$ allele) may be packaged in a phage capsid.
4. Phage with the $A^+$ allele from the donor cell infects a recipient $A^-B^-$ cell, and crossing over (recombination) between donor DNA (brown) and recipient DNA (green) occurs at two places (dotted lines).
5. The genotype of the resulting recombinant cell ($A^+B^-$) differs from the genotypes of both the donor ($A^+B^+$) and the recipient ($A^-B^-$).

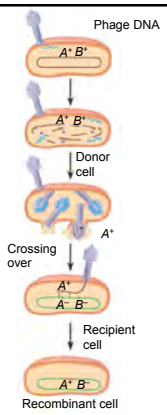
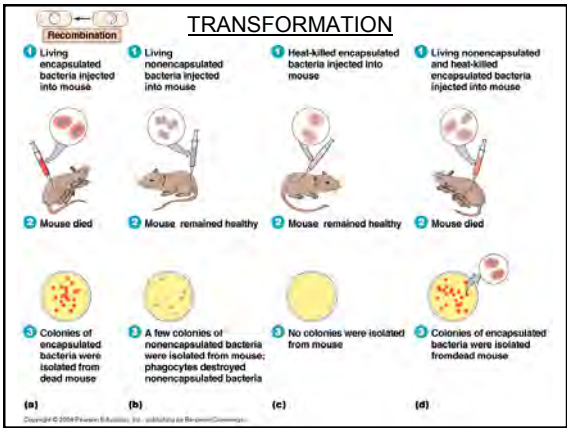
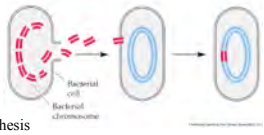



Figure 18.16

## TRANSFORMATION




## TRANSFORMATION

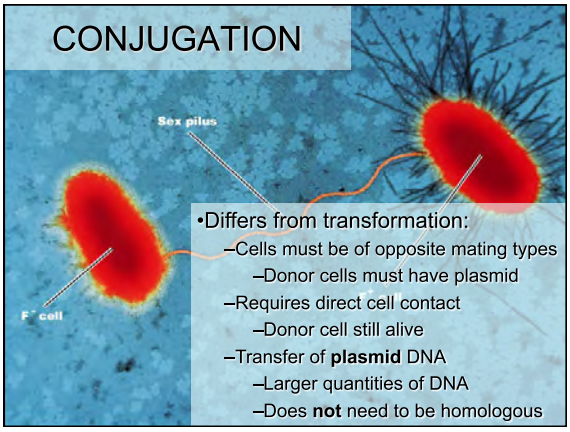


- Cells after death, release DNA
  - Naked DNA in solution
- Cells may take up DNA
  - Only in certain stage of cell cycle
  - Near completion of cell wall synthesis
- Competent cells-receptor sites on cell wall and membrane
- Release competence factor that helps in uptake
  - Alters cell wall
  - Makes it more permeable to DNA
  - *E. coli* needs to be treated to be competent
- Endonucleases cut DNA into small pieces
- Recombination occurs between donor & recipient-complementary base pairing

## Transduction & Transformation

- *In vivo*, restricted to recombination from closely related strains of bacteria
  - Transducing virus must bind and infect both cells.
  - Transforming DNA must be recognized by cell-surface receptors.
  - Donor cell DNA must be homologous to recipient cell DNA for crossing-over.

## CONJUGATION




- Differs from transformation:
  - –Cells must be of opposite mating types
    - –Donor cells must have plasmid
  - –Requires direct cell contact
    - –Donor cell still alive
  - –Transfer of **plasmid** DNA
    - –Larger quantities of DNA
    - –Does **not** need to be homologous

## Plasmids

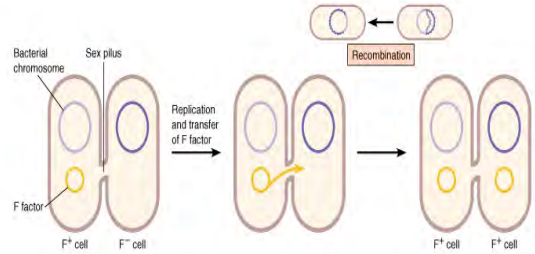
**Extra chromosomal DNA**

- High copy number: many copies per cell
- Low copy number: expression inhibits its own replication

Plasmids grouped by transmissibility:

- **Non-transmissible**
- **Conjugative** — can cause donor cell to initiate contact with recipient cells
  - Carries genes for **sex pili** and for **rolling replication**
- **Mobilizable** — can prepare plasmid DNA for transfer in concert with conjugative plasmid

## F plasmid Conjugation

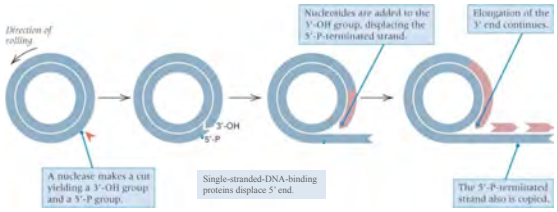



(a) When an F factor (a plasmid) is transferred from a donor ($F^+$) to a recipient ($F^-$), the $F^-$ cell is converted into an $F^+$ cell.

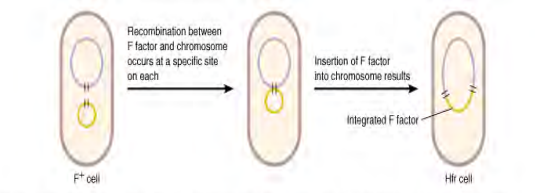


- Donors with F plasmids (F+ cells) transfer plasmid to recipient cells (F-) which become F+

## "**Rolling Replication**" of plasmid DNA



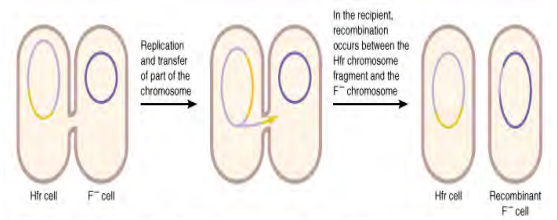

## F plasmid Conjugation




(b) When an F factor becomes integrated into the chromosome of an $F^+$ cell, it makes the cell a high frequency of recombination (Hfr) cell.

- Plasmid codes for proteins allowing insertion into chromosomal DNA
- If plasmid integrates into the chromosome, it converts cell to **Hfr cell** (high frequency of recombination)

## Hfr cell Conjugation




(c) When an Hfr donor passes a portion of its chromosome into an $F^-$ recipient, a recombinant $F^-$ cell results.

- F factor inserted into Hfr chromosome can cause plasmid-like "rolling replication" of large portions of donor chromosomal DNA into recipient cell.
- More DNA that may be recombined into recipient genome.
  - Hence "**H**igh **f**requency of **r**ecombination"!

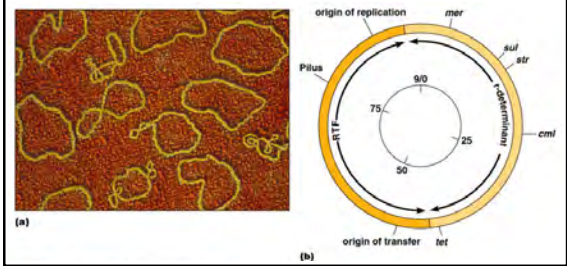
## Plasmids

Extra chromosomal DNA

- **F factor Conjugative plasmids**
  - Carries genes for sex pili (*tra* genes), rolling replication, and for Hfr insertion
- **Dissimilation (metabolic) plasmids**
  - Enzymes that trigger the catabolism of unusual sugars and hydrocarbons
    - *Pseudomonas* use toluene and petroleum as carbon and energy sources
    - **Beneficial-survive in adverse environments**
- **Virulence Plasmids**
  - Convert baterium into a pathogen
    - *E. coli* carries plasmids that code for enterotoxins ➔ diarrhea
- **Col Plasmids**
  - Bacteriocins — toxic proteins kill other bacteria

## R plasmids & Resistance Factors

- **R factors** — resistance to antibiotics, heavy metals or cellular toxins
- Contain two groups of genes
  - **Resistance transfer factor** — genes for plasmid replication and conjugation
  - **R-determinant** has the resistance genes




## HOW BACTERIA GAIN NEW GENETIC INFORMATION

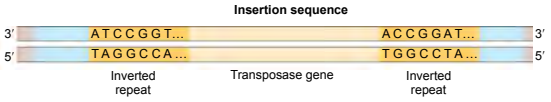
- MUTATIONS
- RECOMBINATION
  - TRANSDUCTION
  - TRANSFORMATION
  - CONJUGATION
- TRANSPOSITION

## *Transposition "Jumping Genes"*

- Rearrangements of large segments of DNA ***within*** the genome
- Mechanism independent of recombination
- Cause deletions, insertions, repetitions, or inversions of DNA
- Often disrupts genes
- May link replicons (sets of genes transcribed together)
- Repetitions cause homologous regions of DNA to occur at different loci in different lineages

## *"Jumping Genes"* — transposable genetic elements

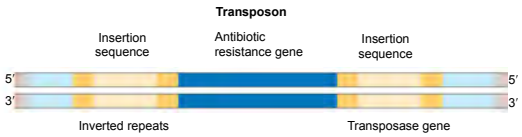
- An **insertion sequence** contains a single gene for **transposase**
  - An enzyme that catalyzes movement of the insertion sequence from one site to another within the genome




**(a)** Insertion sequences, the simplest transposable elements in bacteria, contain a single gene that encodes transposase, which catalyzes movement within the genome. The inverted repeats are backward, upside-down versions of each other; only a portion is shown. The inverted repeat sequence varies from one type of insertion sequence to another.

## *"Jumping Genes"* — transposable genetic elements

- Bacterial **transposons**
  - Also move about within the bacterial genome
  - Have additional genes, such as those for antibiotic resistance




**(b)** Transposons contain one or more genes in addition to the transposase gene. In the transposon shown here, a gene for resistance to an antibiotic is located between twin insertion sequences. The gene for antibiotic resistance is carried along as part of the transposon when the transposon is inserted at a new site in the genome.