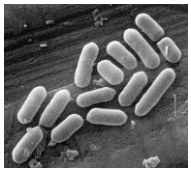
Chapter 18.

Bacterial Genetics

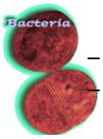
Why study bacterial genetics?

- Its an easy place to start
  - history
  - we know more about it
    - systems better understood
  - simpler genome
  - good model for control of genes
    - build concepts from there to eukaryotes
  - bacterial genetic systems are exploited in biotechnology

Bacteria

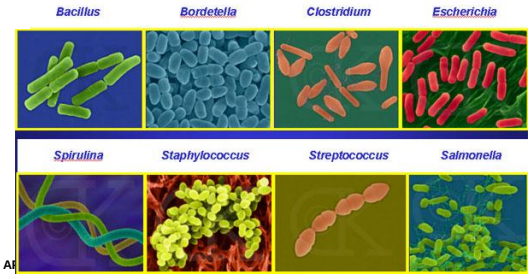


- Bacteria review
  - one-celled organisms
  - prokaryotes
  - reproduce by mitosis
    - binary fission
  - rapid growth
    - generation every ~20 minutes
    - 10^8 colony overnight!
  - dominant form of life on Earth
  - incredibly diverse



Bacterial diversity

rods and spheres and spirals… Oh My!

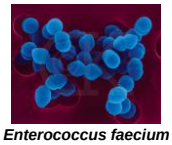


Bacterial diversity

Borrelia burgdorferi
Lyme disease

Treponema pallidum
Syphillis

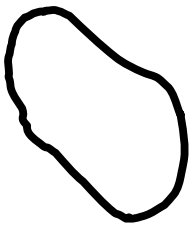
Escherichia coli O157:H7
Hemorrhagic E. coli



Enterococcus faecium
skin infections

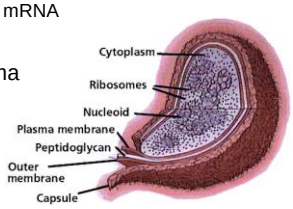
Bacterial genome

- Single circular chromosome
  - haploid
  - naked DNA
    - no histone proteins
  - ~4 million base pairs
    - ~4300 genes
    - 1/1000 DNA in eukaryote



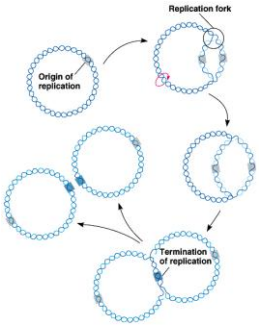
No nucleus!

- No nuclear membrane
  - chromosome in cytoplasm
  - transcription & translation are coupled together
    - no processing of mRNA
  - no introns
  - but Central Dogma still applies
    - use same genetic code



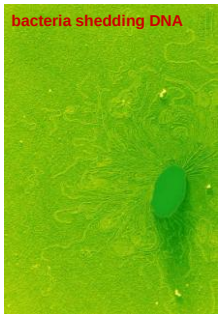
Binary fission

- Replication of bacterial chromosome
- Asexual reproduction
  - offspring genetically identical to parent
  - where does variation come from?



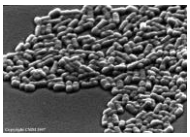
Variation in bacteria

- Sources of variation
  - spontaneous mutation
  - transformation
    - plasmids
    - DNA fragments
  - transduction
  - conjugation
  - transposons



Spontaneous mutation

- Spontaneous mutation is a significant source of variation in <u>rapidly reproducing</u> species
- Example: E. coli
  - human colon
  - 2 x 10^{10} new E. coli each day!
  - spontaneous mutations
    - for 1 gene, only ~1 in 10 million replications
    - each day, ~2,000 bacteria develop mutation in that gene
    - but consider all 4300 genes, then:
      4300 x 2000 = 9 million mutations per day per human host!

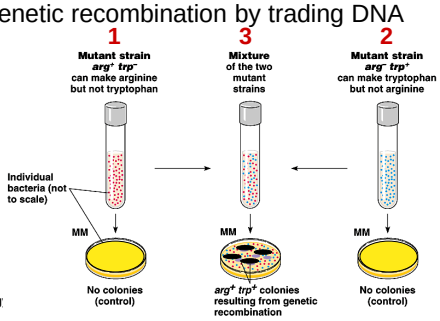


Transformation

- Bacteria are opportunists
  - pick up naked foreign DNA wherever it may be hanging out
    - have surface transport proteins that are specialized for the uptake of naked DNA
  - import bits of chromosomes from other bacteria
  - incorporate the DNA bits into their own chromosome
    - express new gene
    - form of recombination

Swapping DNA

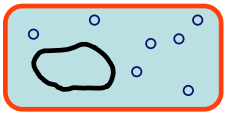
- Genetic recombination by trading DNA



AP Biology

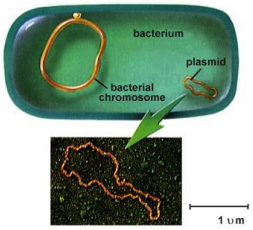
Plasmids

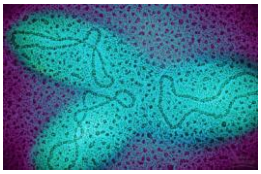
- Plasmids
  - small supplemental circles of DNA
    - 5000 - 20,000 base pairs
    - self-replicating
  - carry extra genes
    - 2-30 genes
  - can be exchanged between bacteria
    - bacterial sex!!
    - rapid evolution
    - antibiotic resistance
  - can be imported from environment



AP Biology

Plasmids

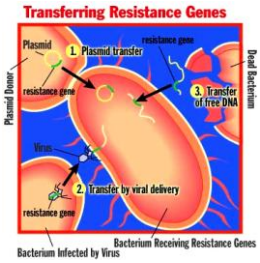




AP Biology

Plasmids & antibiotic resistance

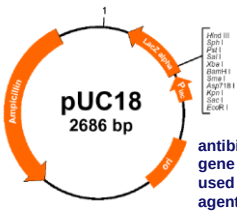
- Resistance is futile?
  - 1st recognized in 1950s in Japan
  - bacterial dysentery not responding to antibiotics
  - worldwide problem now



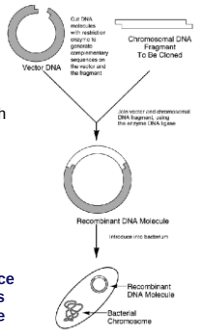
AP Biology

Biotechnology

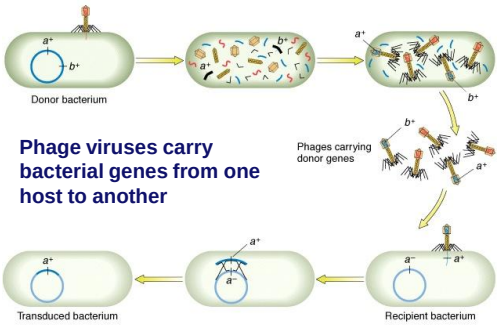
- Used to insert new genes into bacteria
  - example: pUC18
    - engineered plasmid used in biotech



antibiotic resistance gene on plasmid is used as a selective agent



Transduction

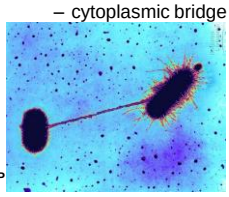


Phage viruses carry bacterial genes from one host to another

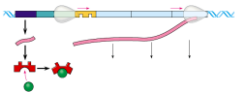
AP

Conjugation

- Direct transfer of DNA between 2 bacterial cells that are temporarily joined
  - results from presence of F plasmid with F factor
    - F for "fertility" DNA
  - E. coli "male" extends sex pilli, attaches to female bacterium
  - cytoplasmic bridge allows transfer of DNA



AP

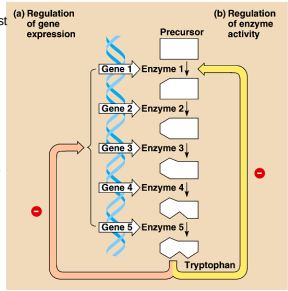


Bacterial Genetics

Regulation of Gene Expression

Regulation of metabolism

- Feedback inhibition
  - product acts as an allosteric inhibitor of 1st enzyme in tryptophan pathway
- Gene regulation
  - block transcription of genes for all enzymes in tryptophan pathway



= inhibition

Gene regulation in bacteria

- Control of gene expression enables individual bacteria to adjust their metabolism to environmental change
- Cells vary amount of specific enzymes by regulating gene transcription
  - turn gene on or turn gene off
    - ex. if you have enough tryptophan in your cell then you don't need to make enzymes used to build tryptophan
      - waste of energy
      - turn off gene which codes for enzymes

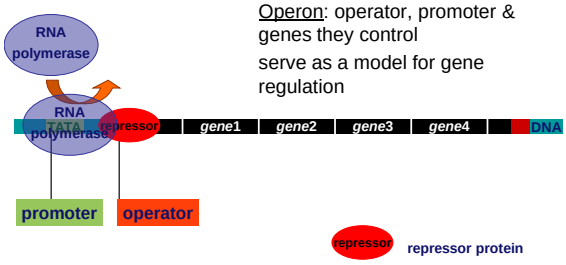
Repressor protein

- So how do you turn off genes?
  - repressor protein binds to DNA near promoter region (TATA box) blocking RNA polymerase
    - binds to operator site on DNA
    - blocks transcription

Repressor protein

Operon: operator, promoter & genes they control

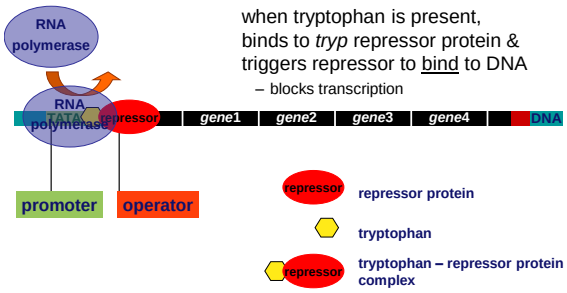
serve as a model for gene regulation



Operons

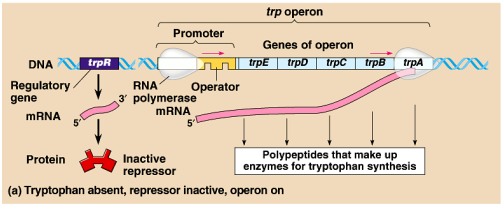
- Bacteria often group together genes with related functions
  - ex. enzymes in a biosynthesis pathway
- Transcription of these genes is controlled by a single promoter
  - when transcribed, read as 1 unit & a single mRNA is made
- Operon
  - operator, promoter & genes they control

Repressible operon: tryptophan



when tryptophan is present, binds to *tryp* repressor protein & triggers repressor to bind to DNA
- blocks transcription

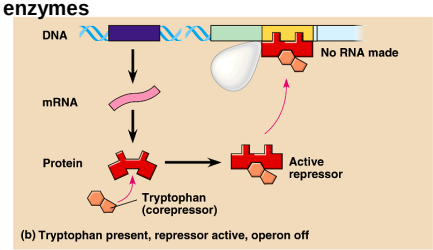
Tryptophan operon



Tryptophan operon

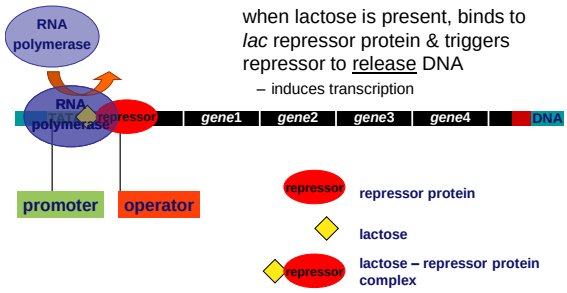
What happens when tryptophan is present?

Don't need to make tryptophan-building enzymes



Tryptophan binds allosterically to regulatory protein

Inducible operon: lactose

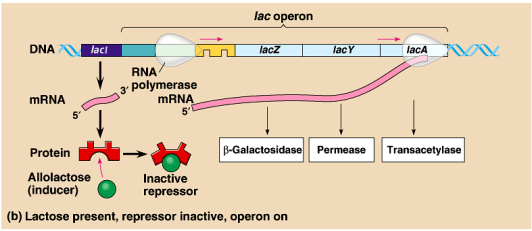


when lactose is present, binds to *lac* repressor protein & triggers repressor to release DNA
- induces transcription

Lactose operon

What happens when lactose is present?

Need to make lactose-digesting enzymes

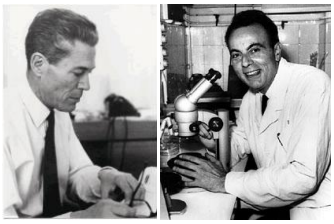


Lactose binds allosterically to regulatory protein

1961|1965

Jacob & Monod: *lac* Operon

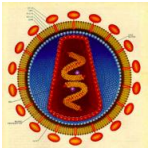
- Francois Jacob & Jacques Monod
  - first to describe operon system
  - coined the phrase "operon"



Jacques Monod **Francois Jacob**

Operon summary

- Repressible operon
  - usually functions in anabolic pathways
    - synthesizing end products
  - when end product is present cell allocates resources to other uses
- Inducible operon
  - usually functions in catabolic pathways,
    - digesting nutrients to simpler molecules
  - produce enzymes only when nutrient is available
    - cell avoids making proteins that have nothing to do

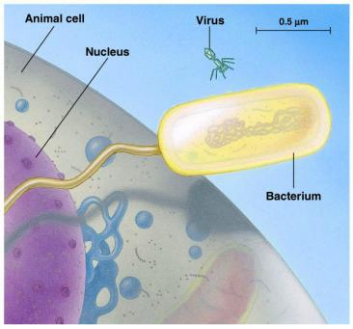


Chapter 18.

Viral Genetics

A sense of size

Comparing
- **eukaryote**
- **bacterium**
- **virus**

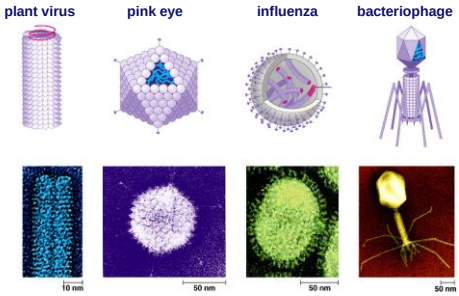


What is a virus? Is it alive?

- DNA or RNA enclosed in a protein coat
- Viruses are not cells
- Extremely tiny
  - electron microscope size
  - smaller than ribosomes
  - ~20–50 nm

1st discovered in plants (1800s)
- **tobacco mosaic virus**
- **couldn't filter out**
- **couldn't reproduce on media like bacteria**

Variation in viruses



a package of genes in transit from 1 host cell to another

Viral genomes

Viral nucleic acid varies
- double-stranded DNA (dsDNA)
- single-stranded DNA (ssDNA)
- double-stranded RNA (dsRNA)
- single-stranded RNA (ssRNA)

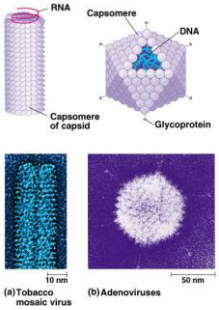
Linear or circular molecule of nucleic acid
- smallest viruses have only 4 genes, while largest have several hundred

Table 18.1 Classes of Animal Viruses, Grouped by Type of Nucleic Acid

Class*	Examples/Diseases
I. dsDNA**	
Papovavirus	Papilloma (human warts, cervical cancer); polyoma (tumors in certain animals)
Adenovirus	Respiratory diseases; some cause tumors in certain animals
Herpesvirus	Herpes simplex I (cold sores), herpes simplex II (genital sores); variolla zoster (chicken pox, shingles); Epstein–Barr virus (mononucleosis, Burkitt's lymphoma)
Poxvirus	Smallpox; vaccinia, cowpox
II. ssDNA	
Parvovirus	Roseola; most parvoviruses depend on co-infection with adenoviruses for growth
III. dsRNA	
Reovirus	Diarrhea; mild respiratory diseases
IV. ssRNA that can serve as mRNA	
Picornavirus	Poliovirus; rhinovirus (common cold); enteric (intestinal) viruses
Togavirus	Rubella virus; yellow fever virus; encephalitis viruses
V. ssRNA that is a template for mRNA	
Rhabdovirus	Rabies
Paramyxovirus	Measles; mumps
Orthomyxovirus	Influenza viruses
VI. ssRNA that is a template for DNA synthesis	
Retrovirus	RNA tumor viruses (*e.g.*, leukemia viruses); HIV (AIDS virus)

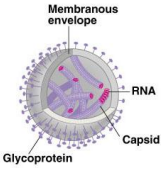
Viral protein coat

- Capsid
  - crystal-like protein shell
  - 1-2 types of proteins
  - many copies of same protein = capsomere

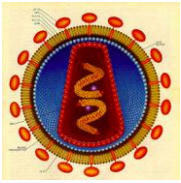


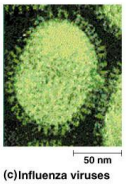
Viral envelope

- Lipid bilayer membranes cloaking viral capsid
  - envelopes are derived from host cell membrane
    - glycoproteins on surface



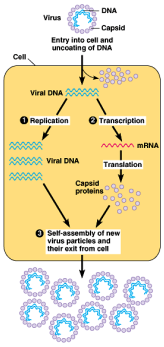
HIV





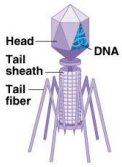
Generalized viral lifecycle

- Parasites
  - lack enzymes for metabolism
  - lack ribosomes for protein synthesis
  - need host "machinery"
- Entry
  - virus DNA/RNA enters host cell
- Assimilation
  - viral DNA/RNA takes over host
  - reprograms host cell to copy viral nucleic acid & build viral proteins
- Self assembly
  - nucleic acid molecules & capsomeres then self-assemble into viral particles
  - exit cell

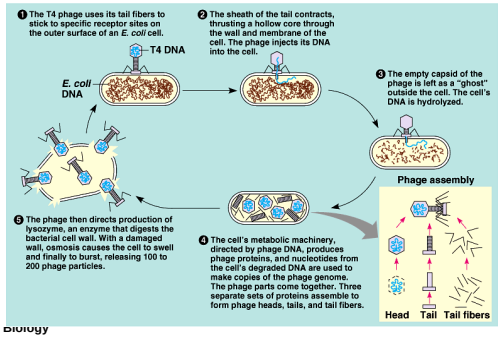


Bacteriophages

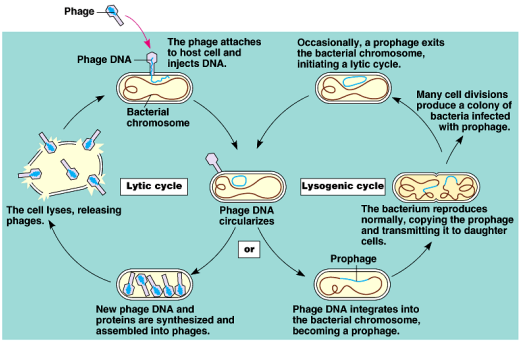
- Viruses that infect bacteria
  - ex. phages that infect *E. coli*
  - 20-sided capsid head encloses DNA
  - protein tail attaches phage to host & injects phage DNA inside



Lytic lifecycle of phages



Lysogenic lifecycle of phages



Viral hosts

- Host range
  - each type of virus can infect & parasitize only a limited range of host cells
  - identify host cells via "lock & key" fit
    - between proteins on viral coat & receptors on host cell surface
  - broad host range
    - rabies = can infect all mammals
  - narrow host range
    - human cold virus = only cells lining upper respiratory tract of humans
    - AIDS virus = binds only to specific white blood cells

Defense against viruses

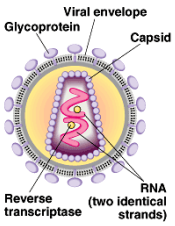
- Bacteria have defenses against phages
  - natural selection favors bacterial mutants with receptors sites that are no longer recognized by a particular type of phage
  - bacteria produce restriction enzymes that recognize & cut up foreign DNA
    - modifications to bacteria's own DNA prevent its destruction by restriction enzymes
- It's an escalating war!
  - natural selection favors phage mutants resistant to the bacterial defenses

RNA viruses

- Retroviruses
  - use an enzyme = reverse transcriptase
  - copies viral RNA into DNA in host
    - viral DNA can be integrated into host chromosome
    - can be passed on to other cells
  - host's RNA polymerase now transcribes viral DNA into viral RNA molecules
    - produces viral components

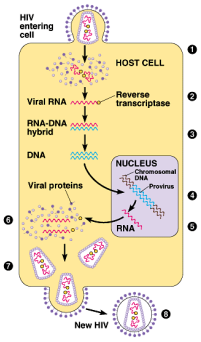
Retroviruses

- HIV
  - human immunodeficiency virus
  - causes AIDS
    - acquired immunodeficiency syndrome
  - envelope with glyco-proteins for binding to specific WBC
  - capsid containing 2 RNA strands & 2 copies of reverse transcriptase



HIV infection

- HIV enters host cell
  - reverse transcriptase synthesizes double stranded DNA from viral RNA
- Transcription produces more copies of viral RNA
  - translated into viral proteins
  - proteins & vRNA self-assemble into virus particles & leave host



Symptoms of viral infection

- Link between infection & symptoms varies
  - kill cells by lysis
  - cause infected cell to produce toxins
  - viral components, such as envelope proteins, may be toxic
- Damage?
  - depends…
    - lung epithelium after the flu is repaired
    - nerve cell damage from polio is permanent

Cancer viruses

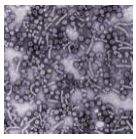
- Viruses appear to cause certain human cancers
  - hepatitis B virus
    - linked to liver cancer
  - Epstein-Barr virus = infectious mononucleosis
    - linked to Burkitt's lymphoma
  - Papilloma viruses
    - linked with cervical cancers
  - HTLV-1 retrovirus
    - linked to type of adult leukemia

Cancer viruses

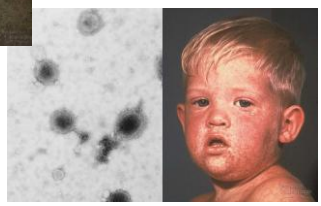
- Transform cells into cancer cells after integration of viral DNA into host DNA
  - carry oncogenes that trigger cancerous characteristics in cells
  - version of human gene that normally controls cell cycle or cell growth
- Most tumor viruses probably cause cancer only in combination with other mutagenic events

Viral diseases

Polio

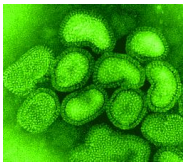


Hepatitis



Measles

Influenza: 1918 epidemic

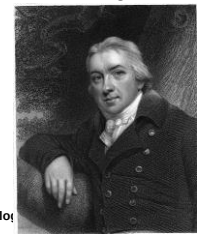


RNA virus

30-40 million deaths world-wide

Vaccines

- Injections of harmless variants of virus
  - stimulate immune system to mount rapid defense against future attack



Edward Jenner
1st vaccine

And there's more

Prions

- Spongiform encephalopathies
  - misfolded versions of normal brain proteins
    - induce normal proteins to take on abnormal shape
    - destroy brain cell & brain function
    - mad cow disease
    - Creutzfeldt-Jakob disease