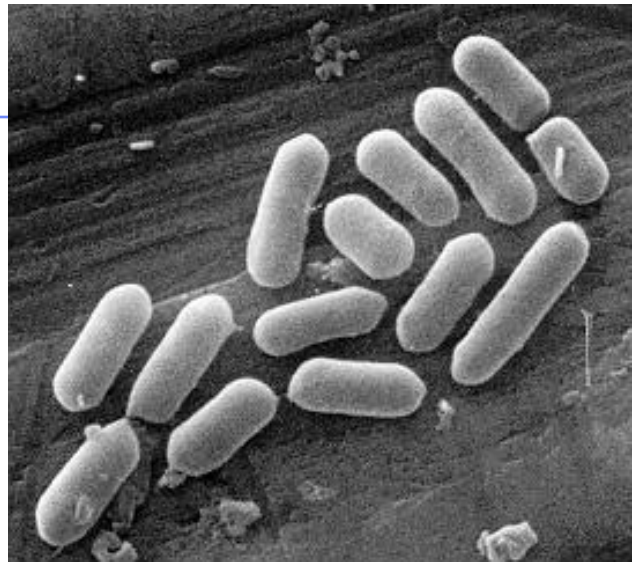
Chapter 18.

The Exciting World Of Bacterial Genetics

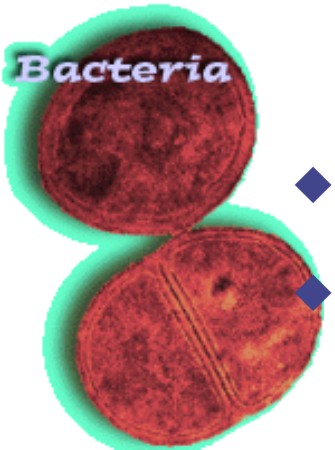
Why study bacterial genetics?

- 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

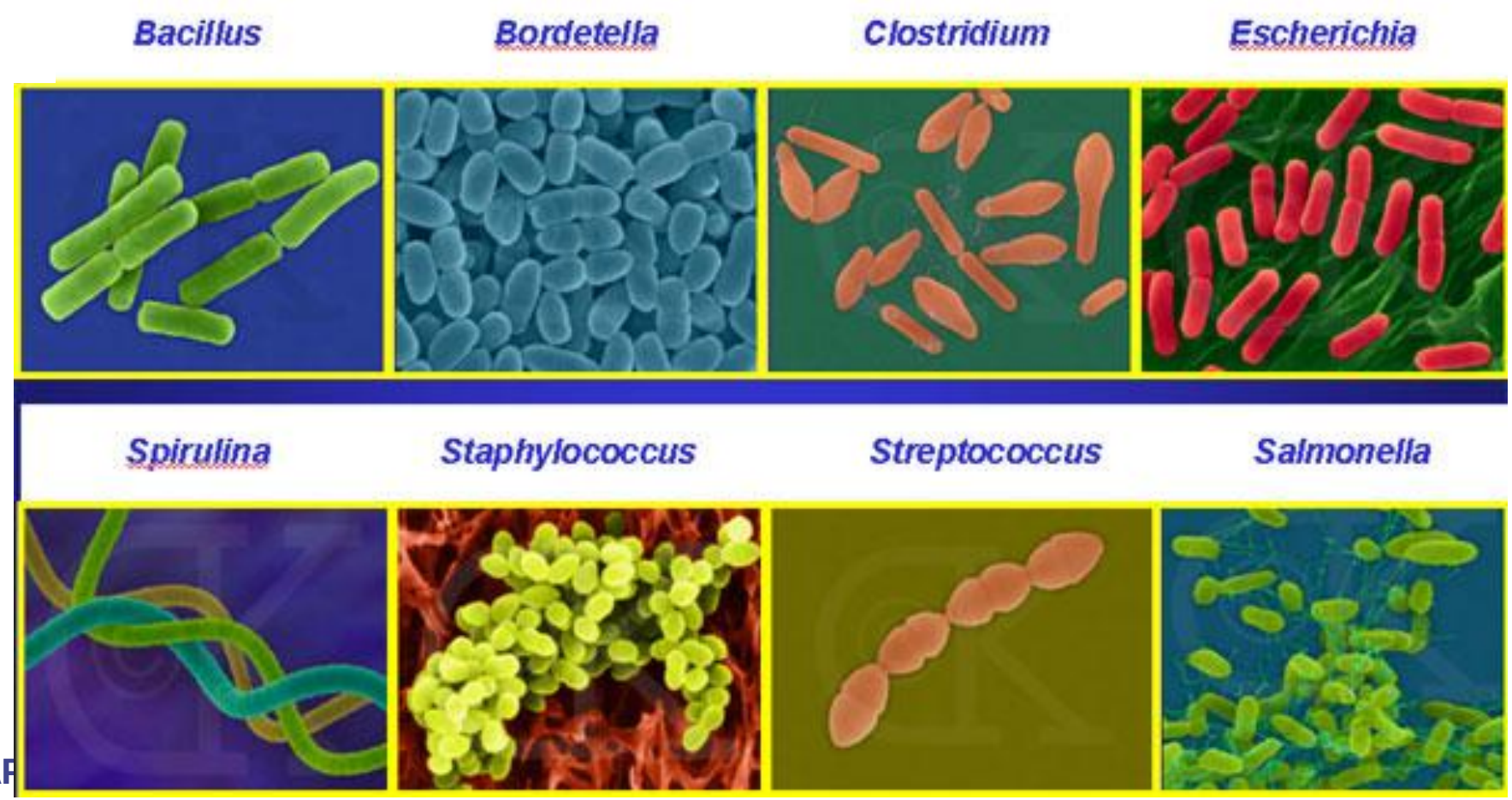


- Bacteria review
  - one-celled organisms
  - prokaryotes
  - reproduce by mitosis
    - binary fission
  - rapid growth
    - generation every ~20 minutes
    - 10^8 (100 million) colony overnight!
  - dominant form of life on Earth
  - incredibly diverse

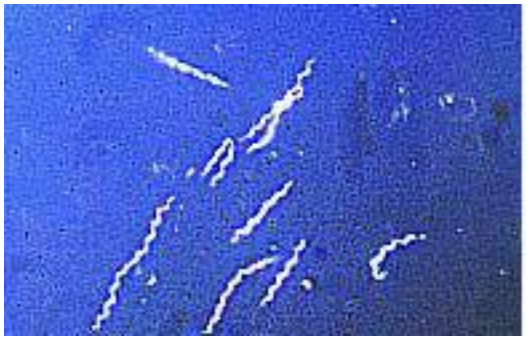


Bacterial diversity

rods and spheres and spirals… Oh My!



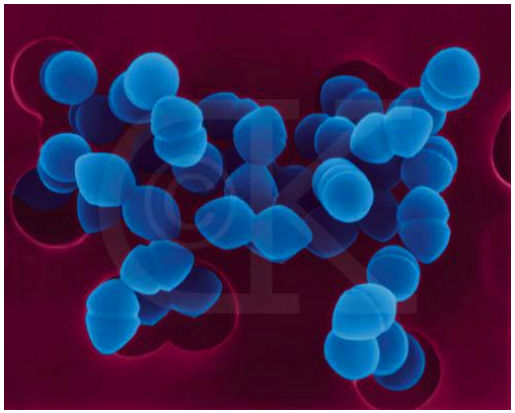
Bacterial diversity



Borrelia burgdorferi
Lyme disease

Treponema pallidum
Syphillis

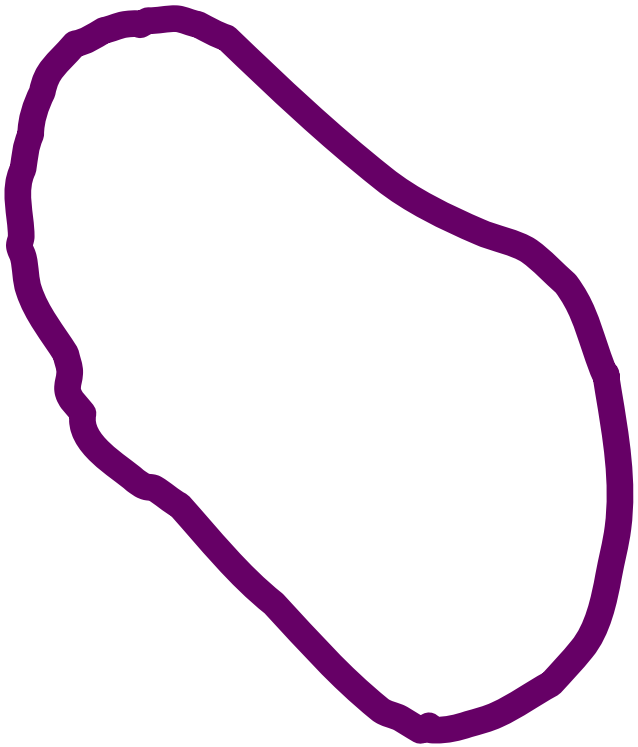
Escherichia coli O157:H7
Hemorrhagic E. coli



Enterococcus faecium
skin infections

Bacterial genome

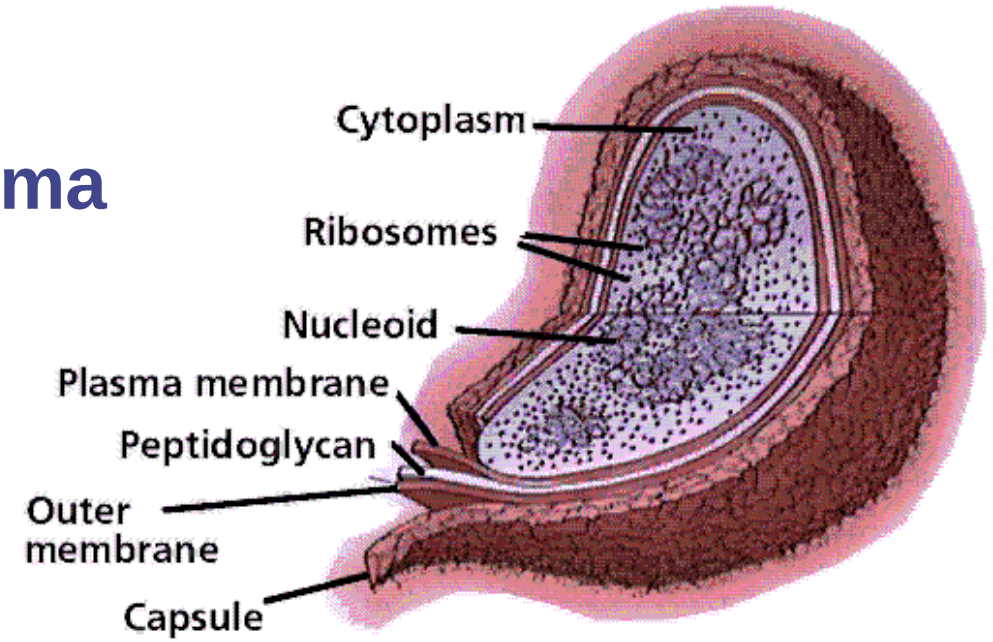
- Single circular chromosome
  - haploid
  - naked DNA
    - no histone proteins
  - ~4 million base pairs
    - ~4300 genes
    - 1/1000 DNA in eukaryote



Intro to Bacteria video

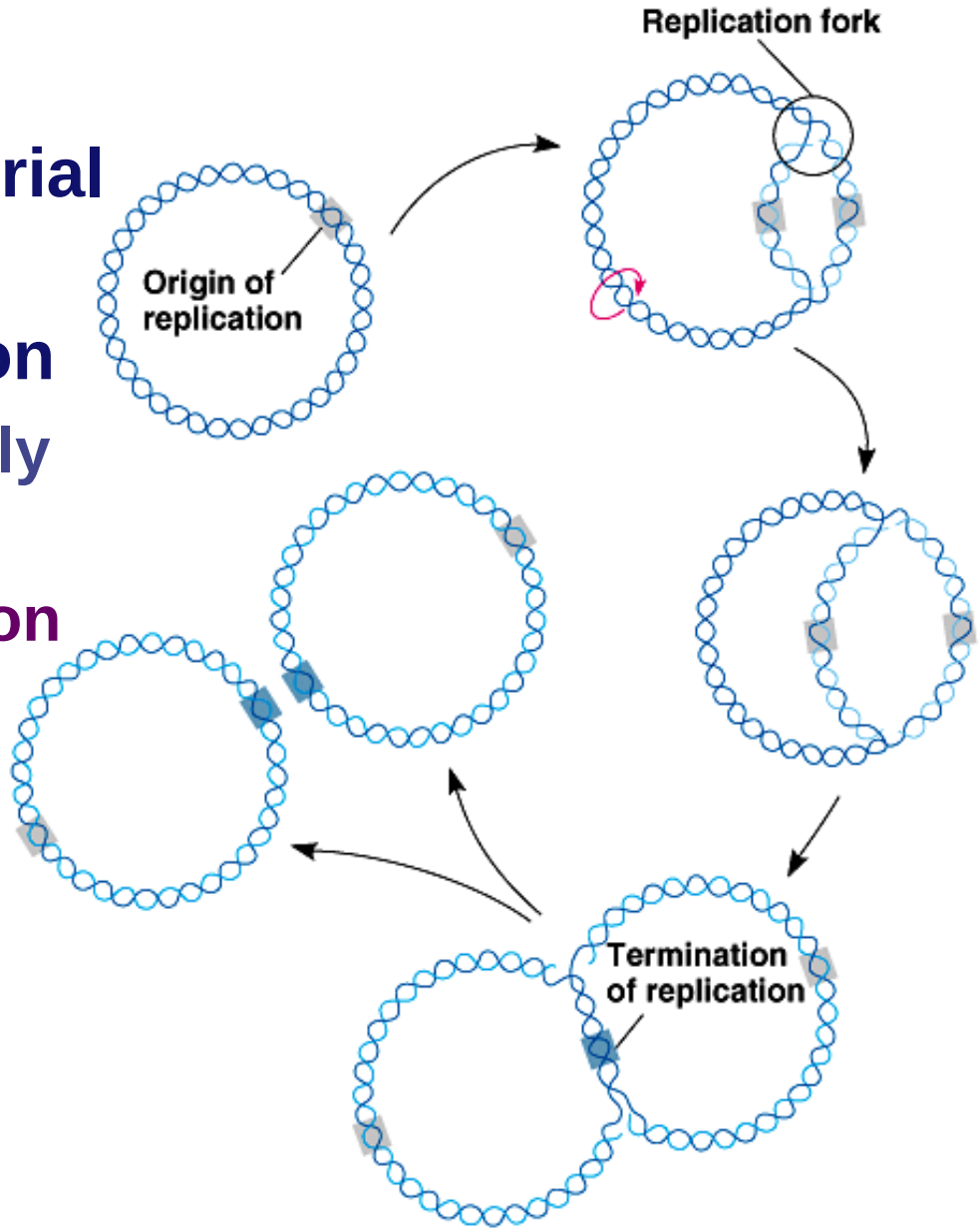
No nucleus!

- 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



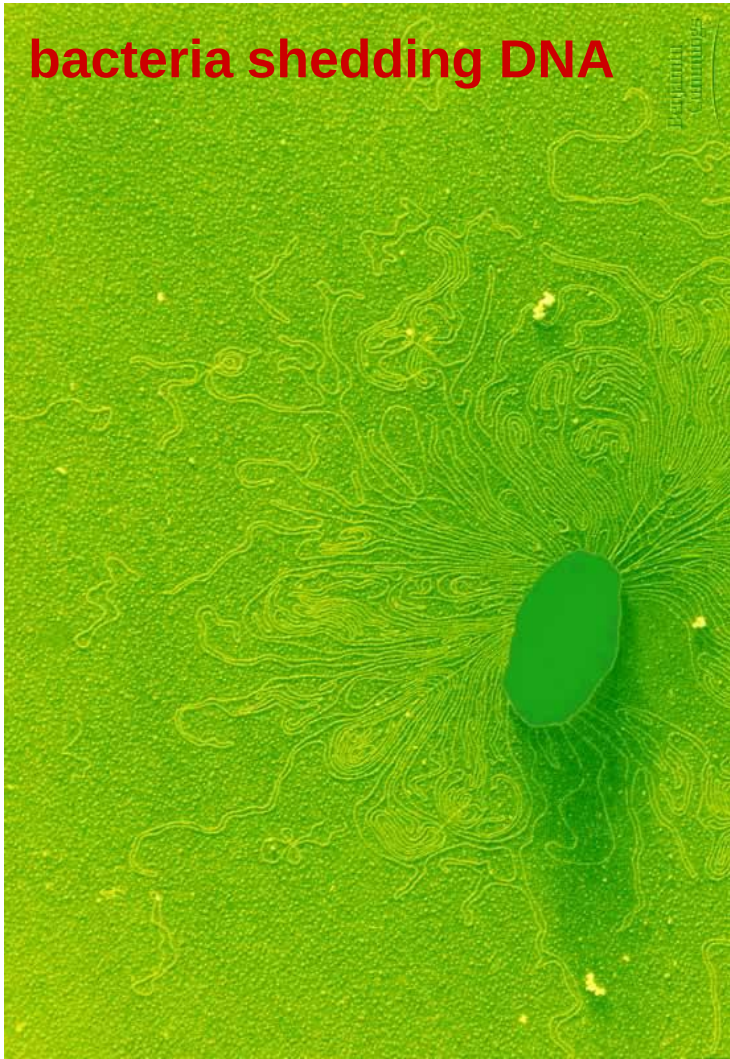
Binary fission

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

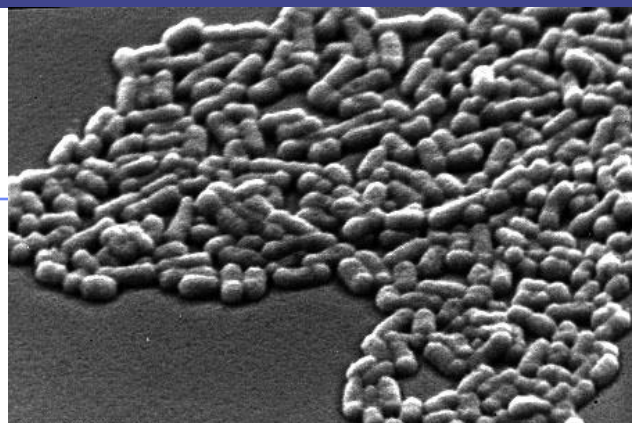


Variation in bacteria

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



Spontaneous mutation



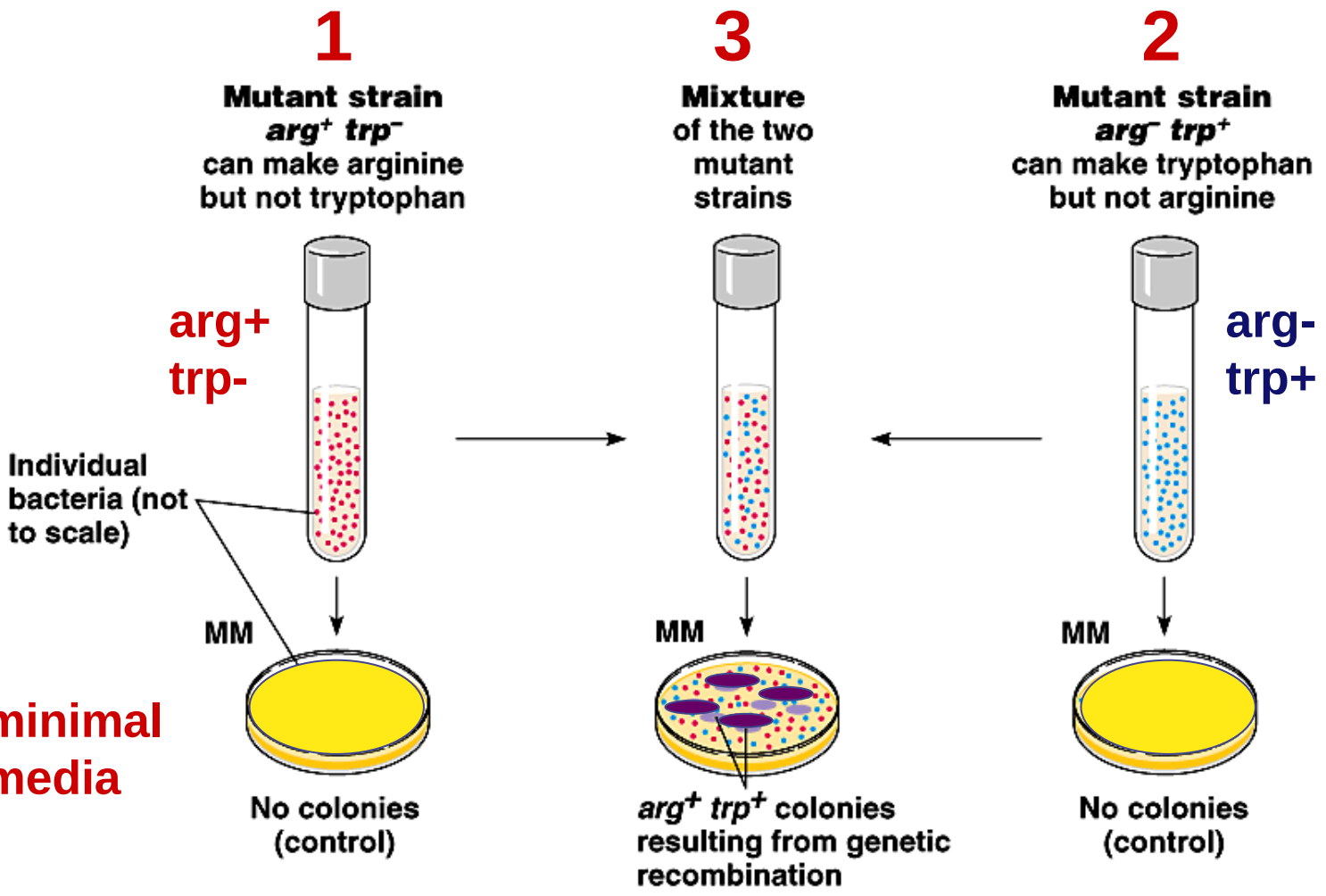
- Spontaneous mutation is a significant source of variation in rapidly reproducing species
- Example: E. coli
  - human colon (large intestines)
  - 2 x 10^{10} (billion) new E. coli each day!
  - spontaneous mutations
    - for 1 gene, only ~1 mutation in 10 million replications
    - each day, ~2,000 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**

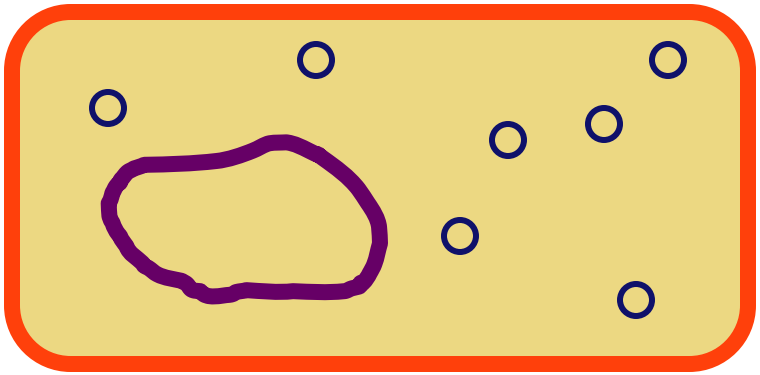
Swapping DNA

- Genetic recombination by trading DNA

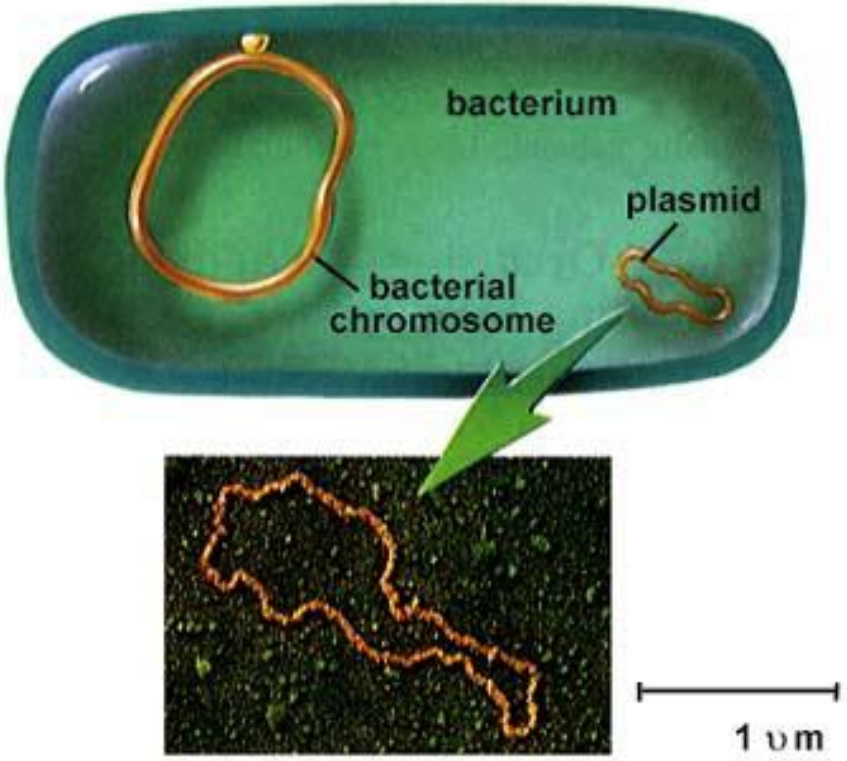


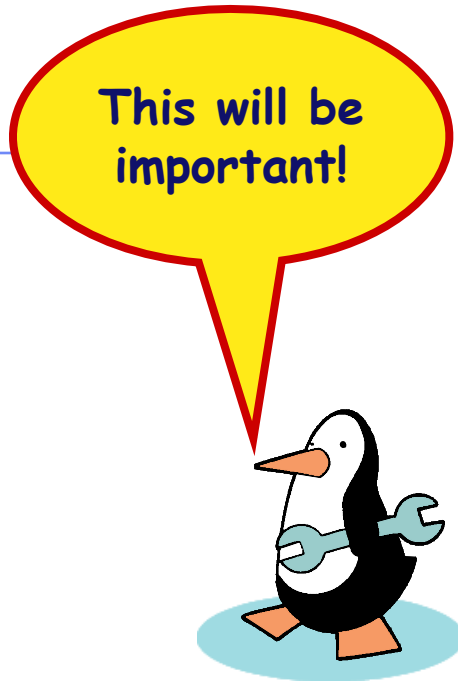
Plasmids

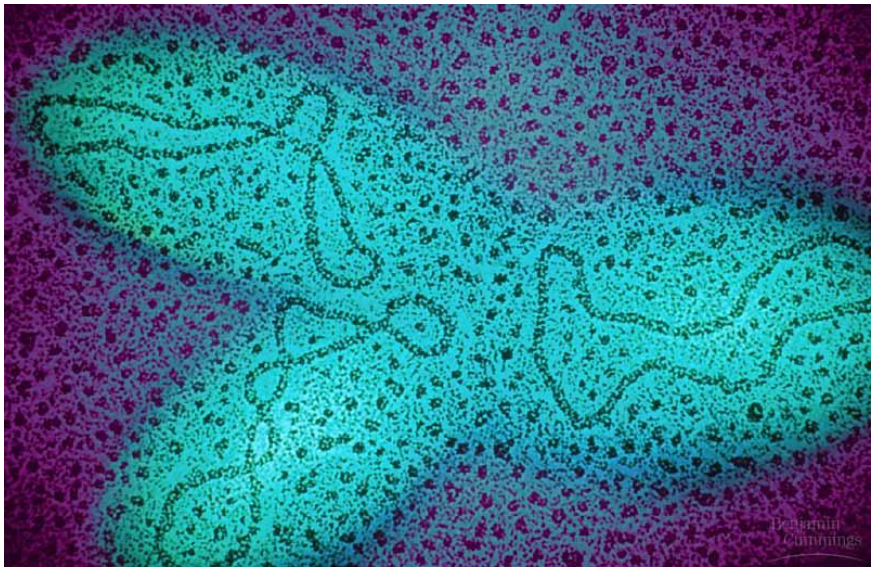
- **Plasmids**
  - small supplemental circles of DNA
    - 5000 - 20,000 base pairs
    - self-replicating
  - carry extra genes
    - 2-30 genes
  - can be exchanged between bacteria
    - bacterial sex!!
    - rapid evolution
    - antibiotic resistance
  - can be imported from environment



Plasmids

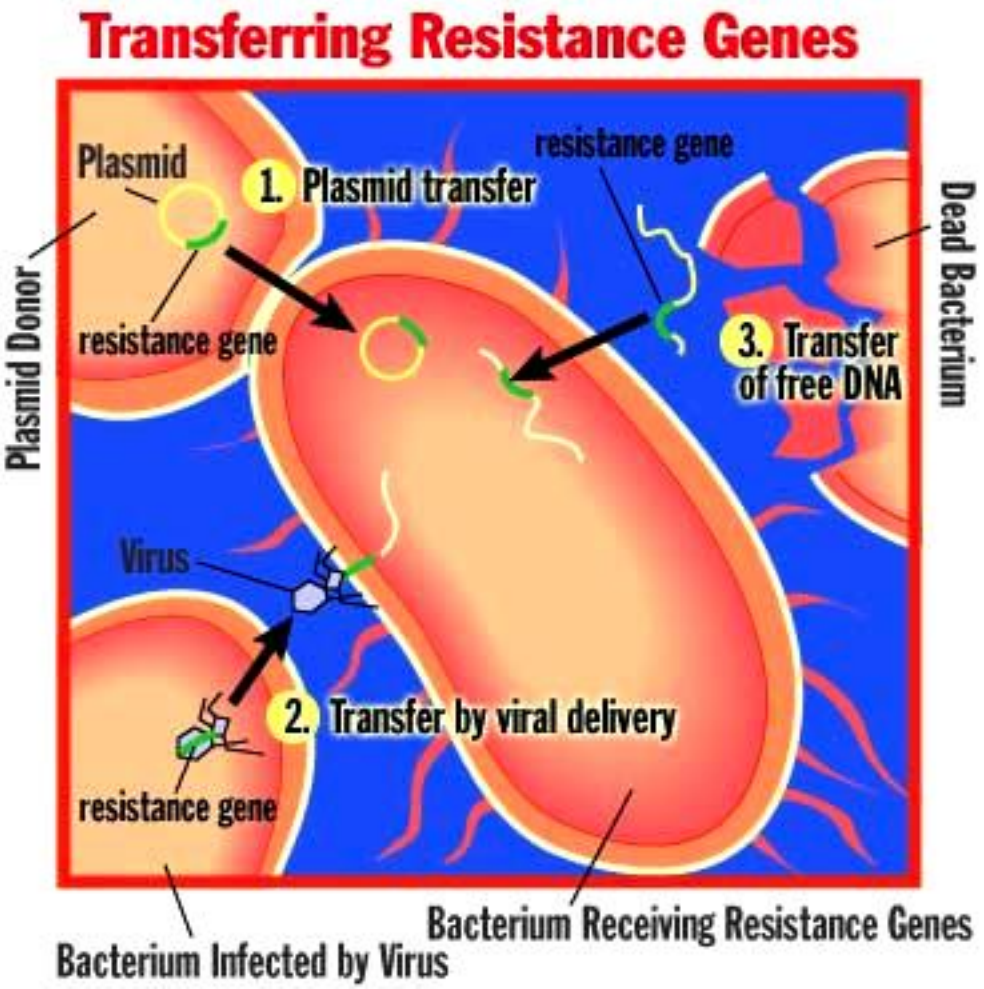

bacterium
plasmid
bacterial chromosome
1 μm


This will be important!



Plasmids & antibiotic resistance

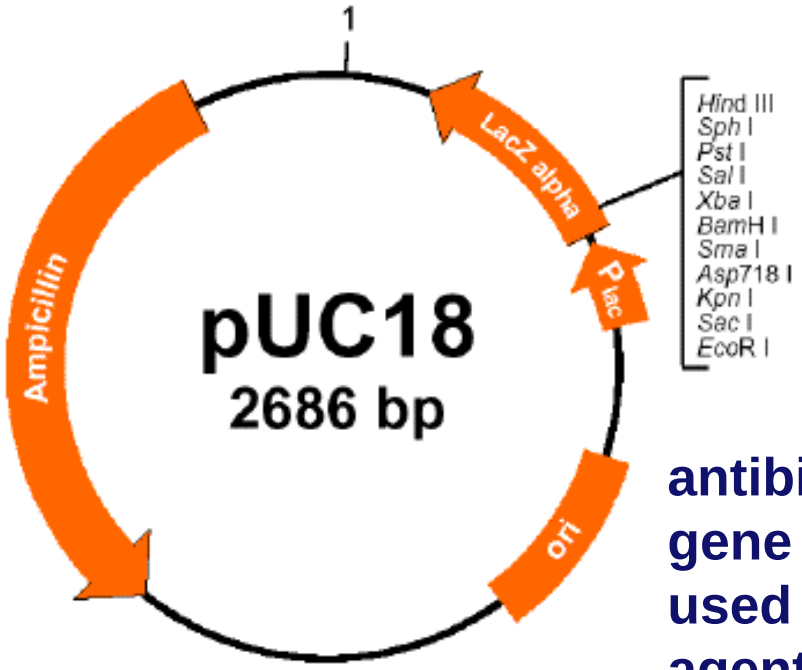
- **Resistance is futile?**
  - 1st recognized in 1950s in Japan
  - bacterial dysentery not responding to antibiotics
  - worldwide problem now
    - resistant genes are on plasmids that are swapped between bacteria



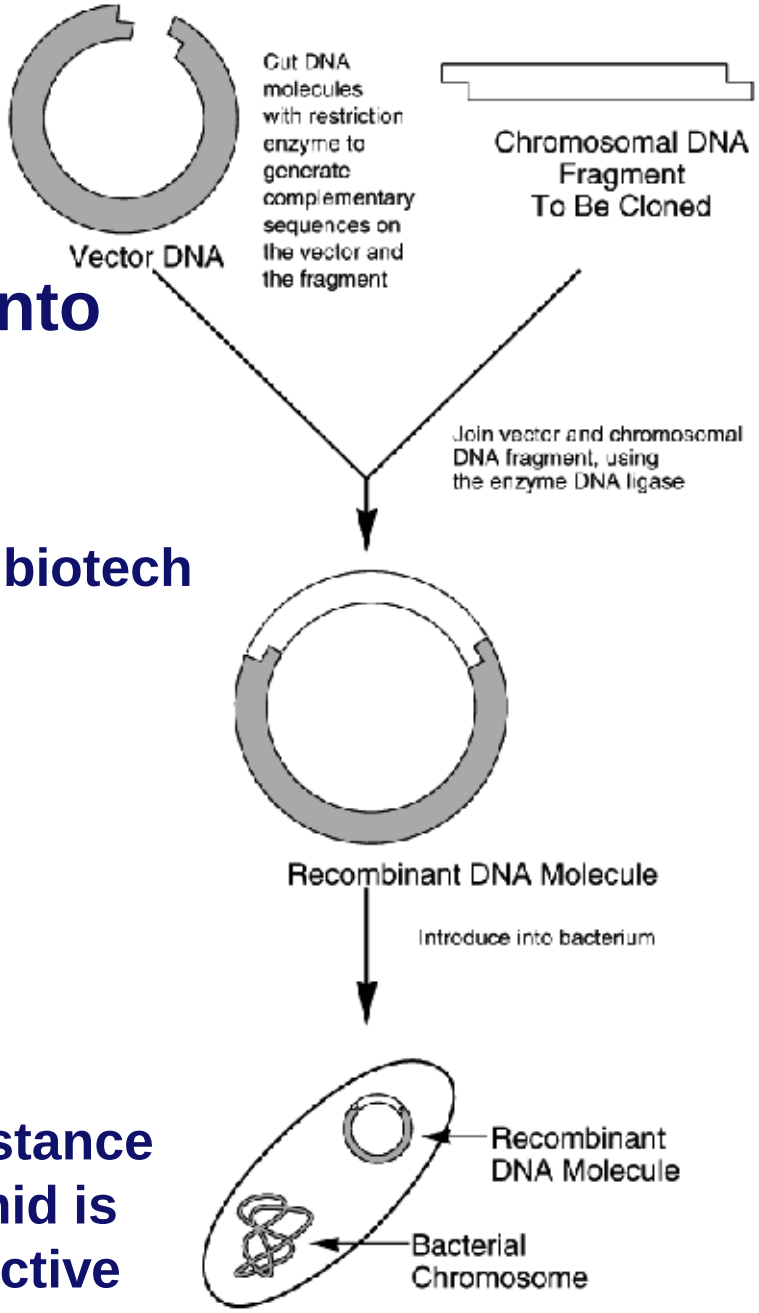
Resistance in Bacteria video

Biotechnology

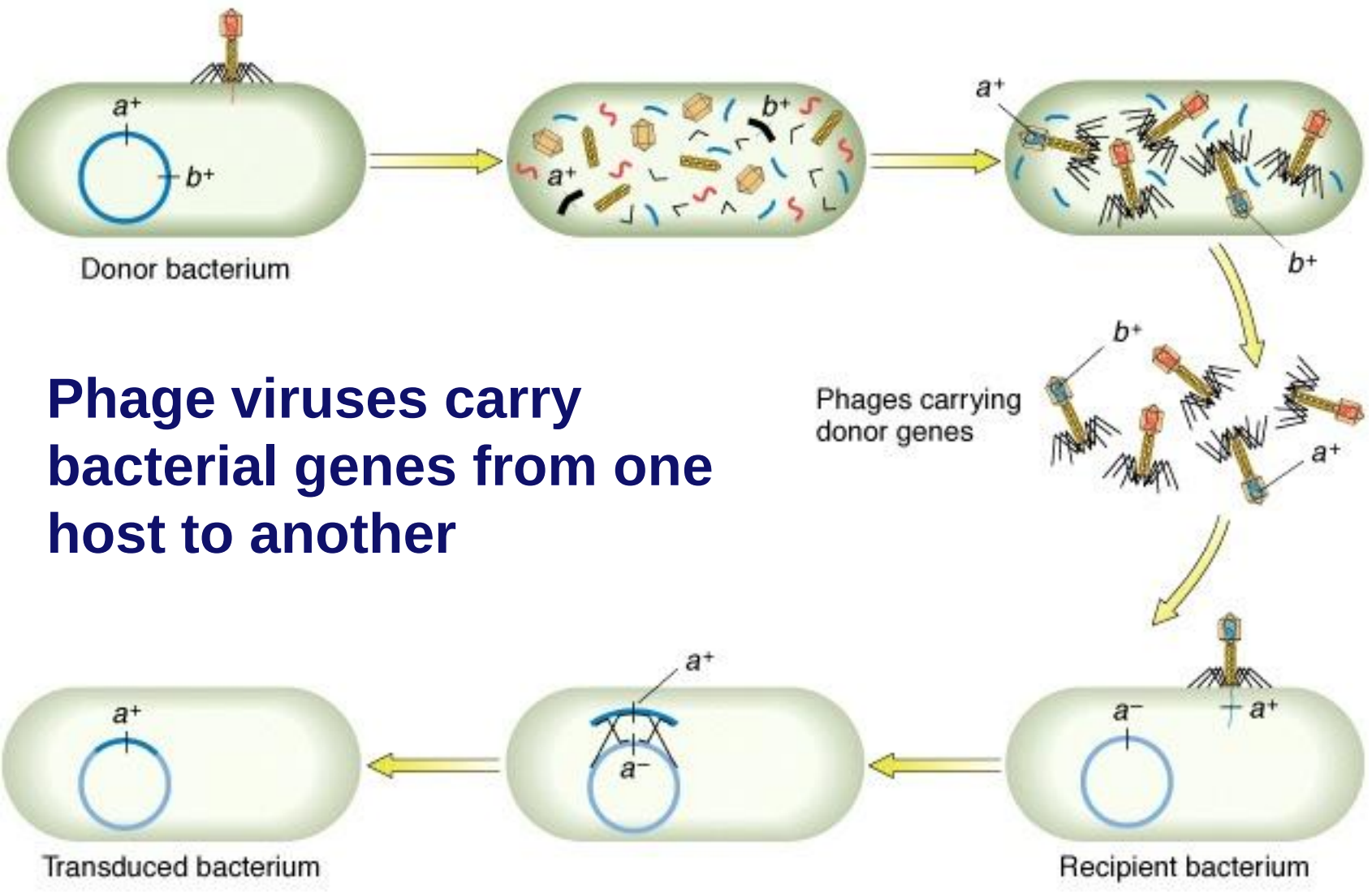
- Used to insert new genes into bacteria
  - example: pUC18
    - engineered plasmid used in biotech



antibiotic resistance gene on plasmid is used as a selective agent



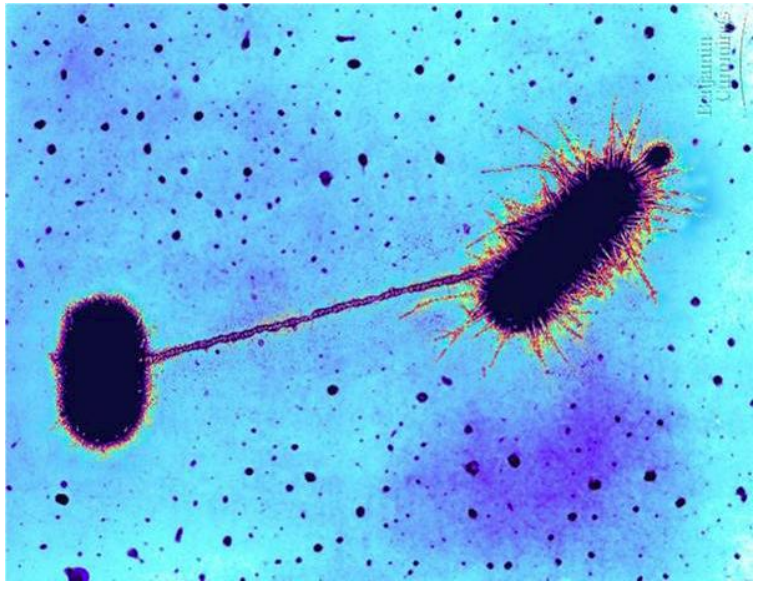
Transduction



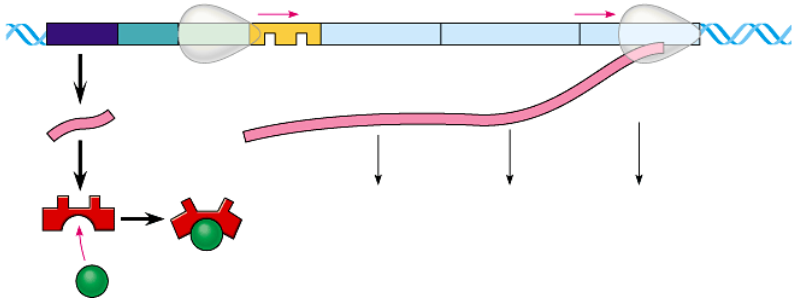
Phage viruses carry bacterial genes from one host to another

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

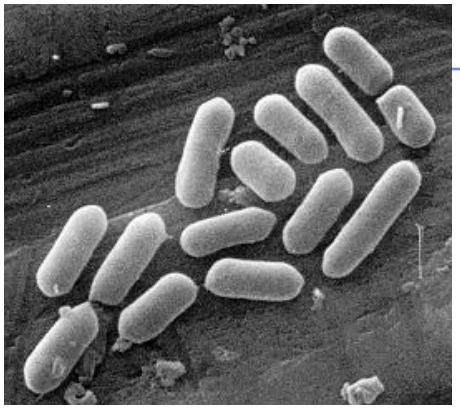


Any Questions??



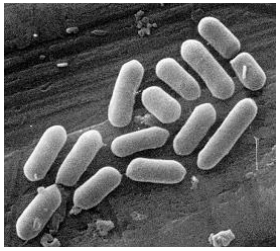
Bacterial Genetics

Regulation of Gene Expression



2005-2006

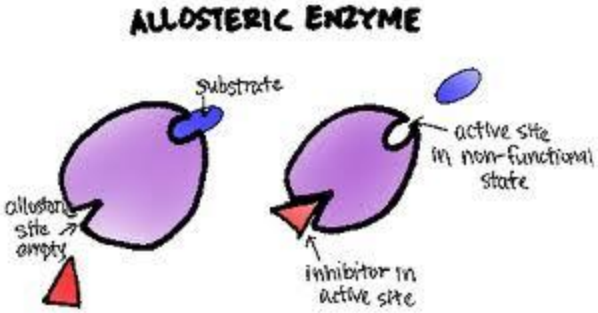
Bacterial metabolism



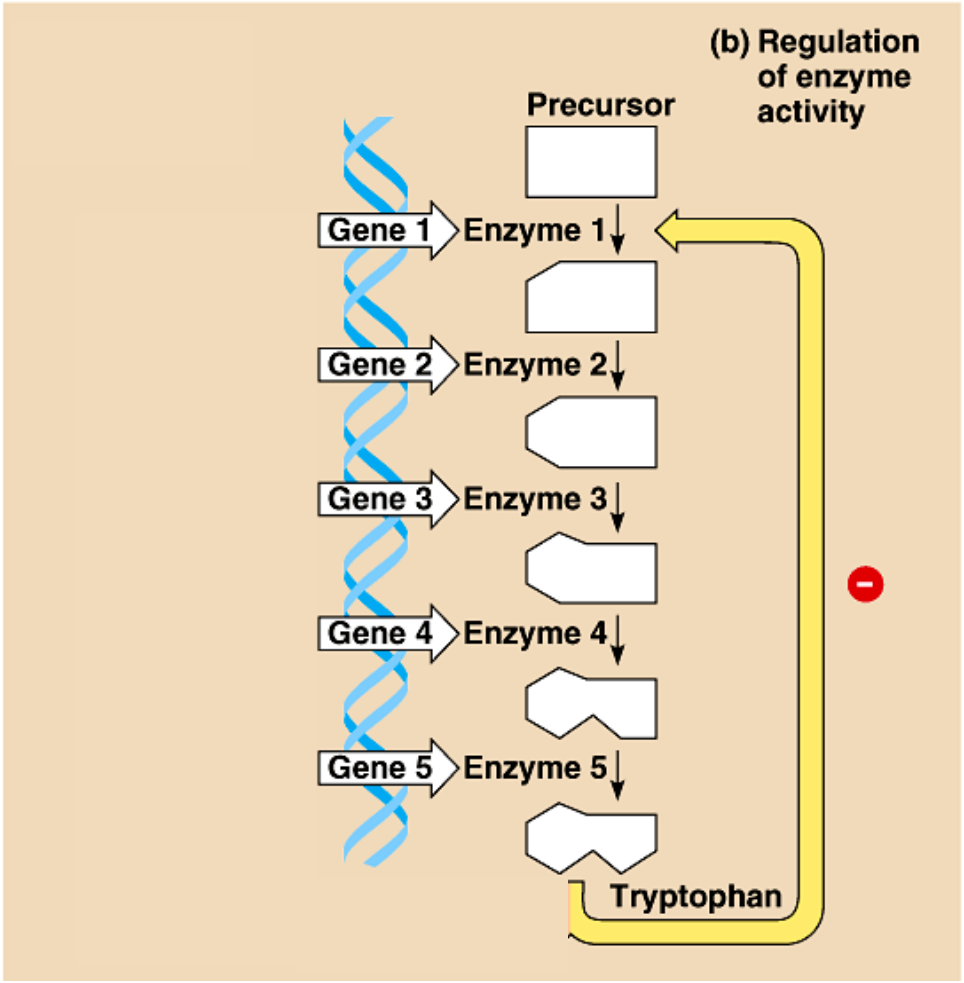
- Bacteria need to respond quickly to changes in their environment
  - if have enough of a product, need to stop production
    - why? waste of energy to produce more
    - how? stop production of synthesis enzymes
  - if find new food/energy source, need to utilize it quickly
    - why? metabolism, growth, reproduction
    - how? start production of digestive enzymes

Reminder: Regulation of metabolism

- **Feedback inhibition**
  - **product acts as an allosteric inhibitor of 1st enzyme in tryptophan pathway**

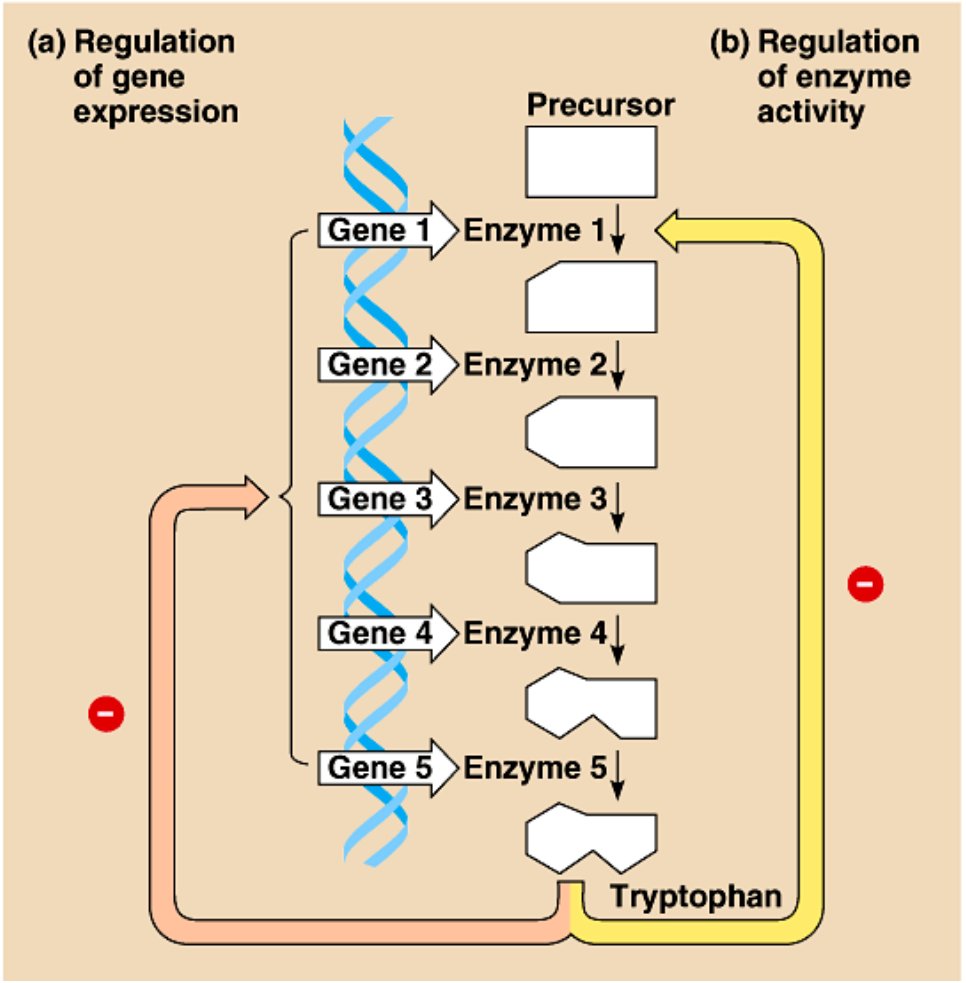


= inhibition



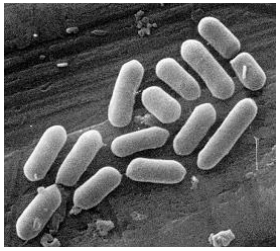
Another way to Regulate metabolism

- Gene regulation
  - block transcription of genes for all enzymes in tryptophan pathway
    - saves energy by not wasting it on unnecessary protein synthesis



= inhibition

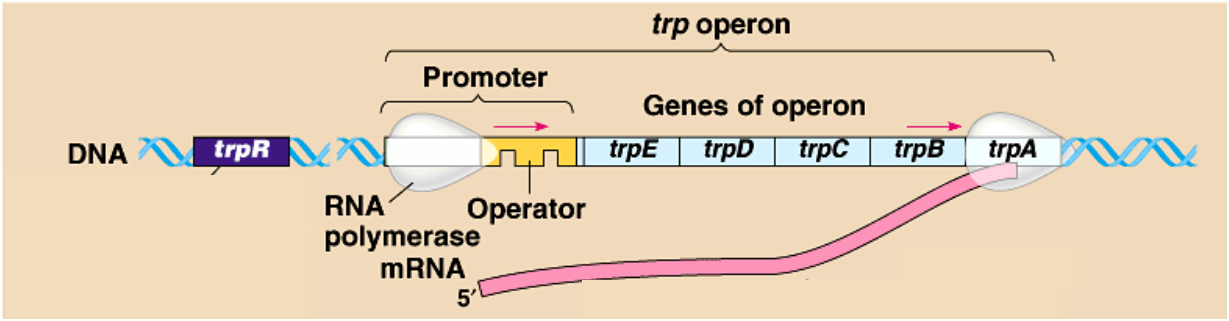
Gene regulation in bacteria



- 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 genes on or turn genes 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 genes which codes for enzymes

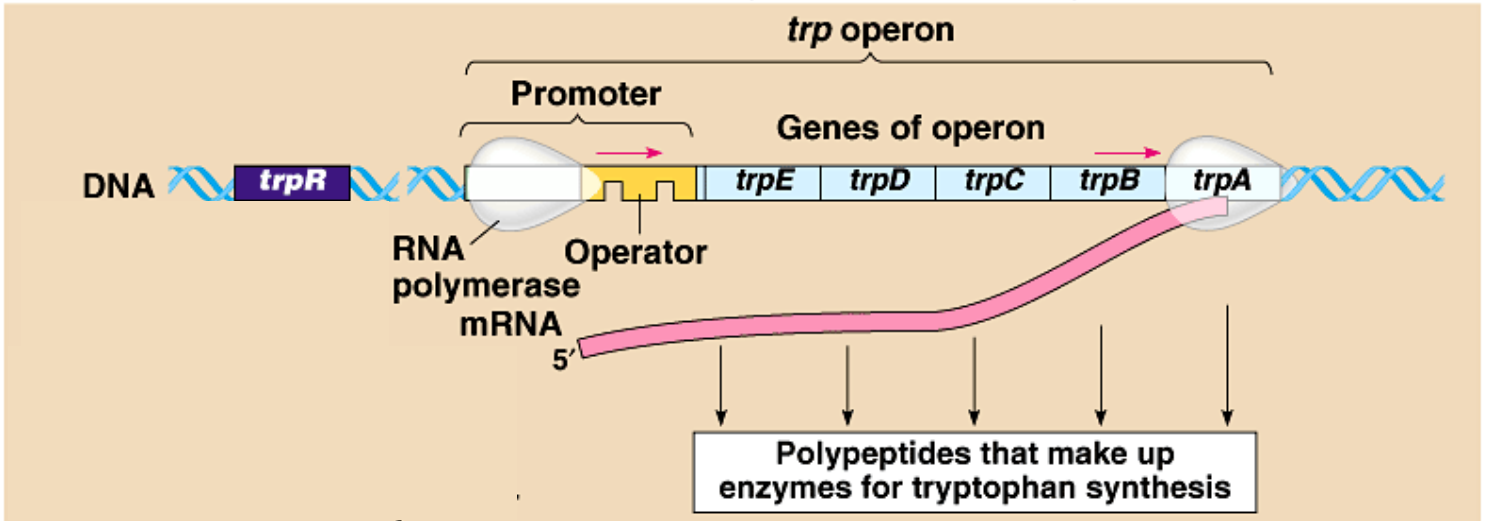
So how can genes be turned off?

- First step in protein production?
  - transcription
  - stop RNA polymerase!
- Repressor protein
  - binds to DNA near promoter region blocking RNA polymerase
    - binds to operator site on DNA
    - blocks transcription



Genes grouped together

- Operon
  - genes grouped together with related functions
    - ex. enzymes in a synthesis pathway
  - promoter = RNA polymerase binding site
    - single promoter controls transcription of all genes in operon
    - transcribed as 1 unit & a single mRNA is made
  - operator = DNA binding site of regulator protein

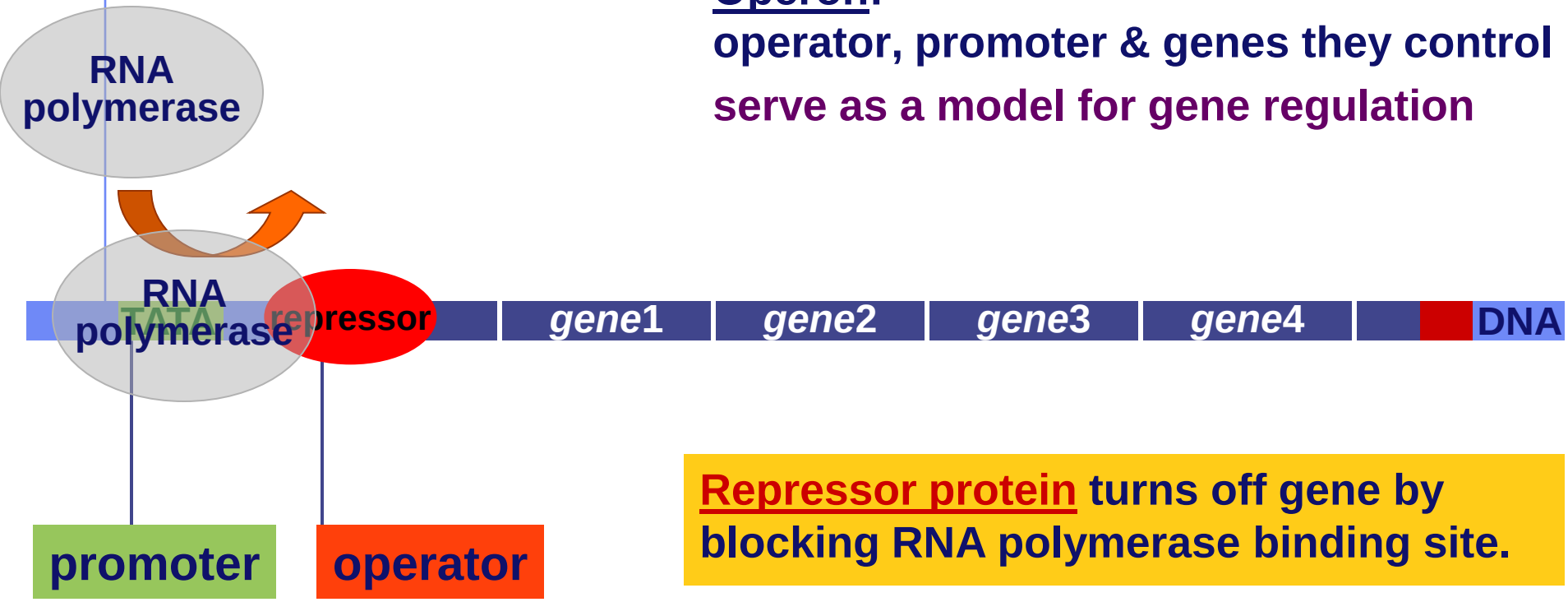


Monday, April 8th:

What does the repressible operon model remind you of?

Repressor protein model

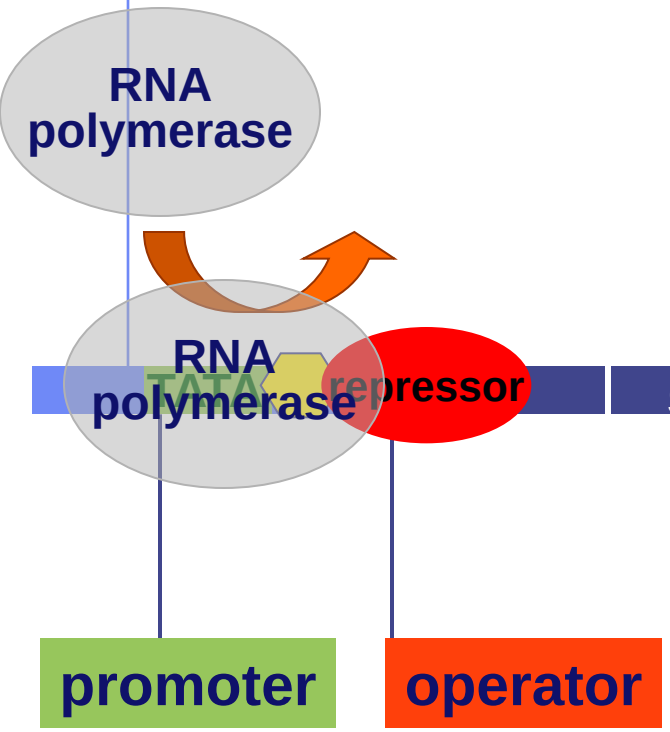
Operon:
operator, promoter & genes they control
serve as a model for gene regulation



Repressor protein turns off gene by blocking RNA polymerase binding site.

repressor — repressor protein

Repressible operon: tryptophan



Synthesis pathway model

When excess tryptophan is present, binds to *tryp* repressor protein & triggers repressor to bind to DNA

- **blocks (represses) transcription**

repressor protein

tryptophan

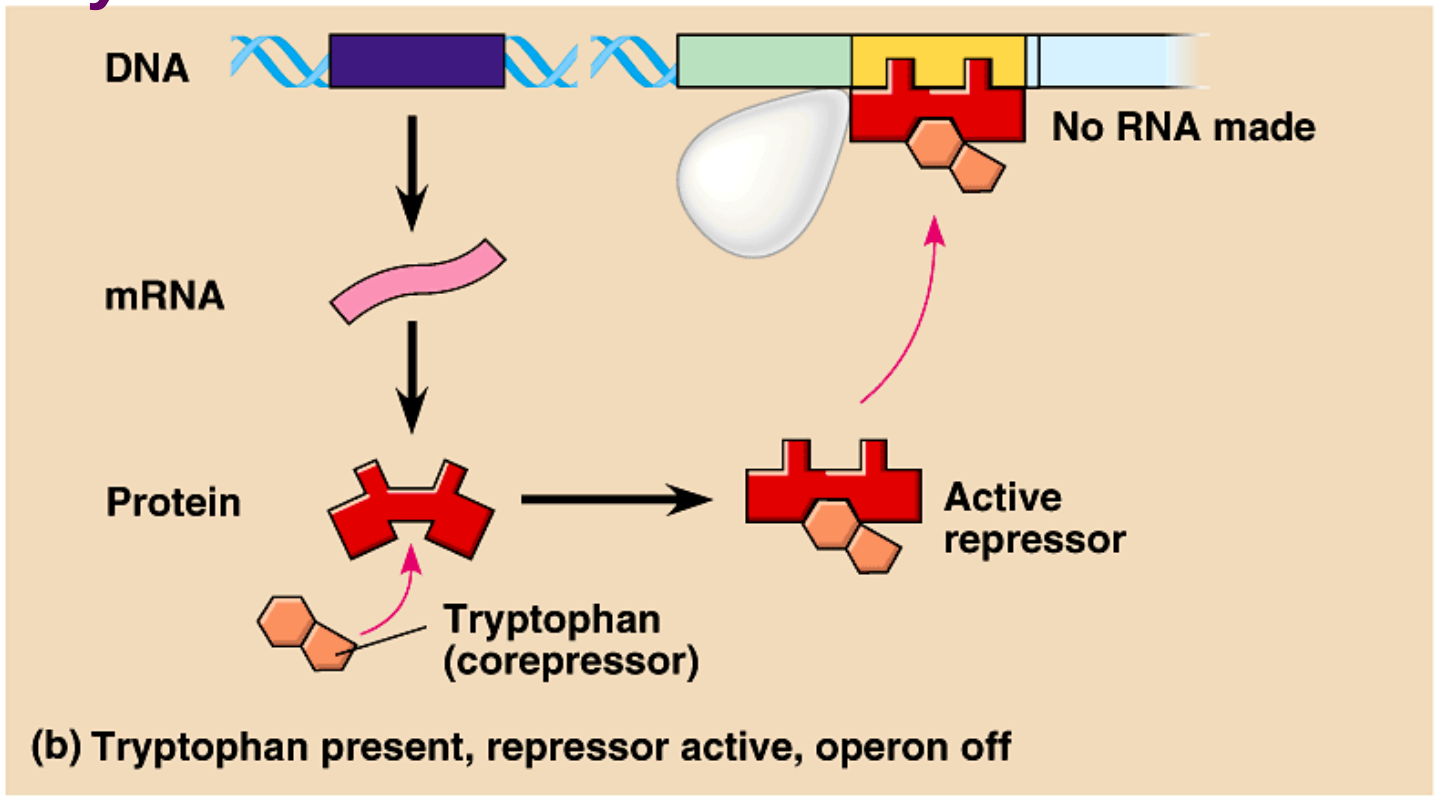
tryptophan – repressor protein complex

conformational change in repressor protein!

Tryptophan operon

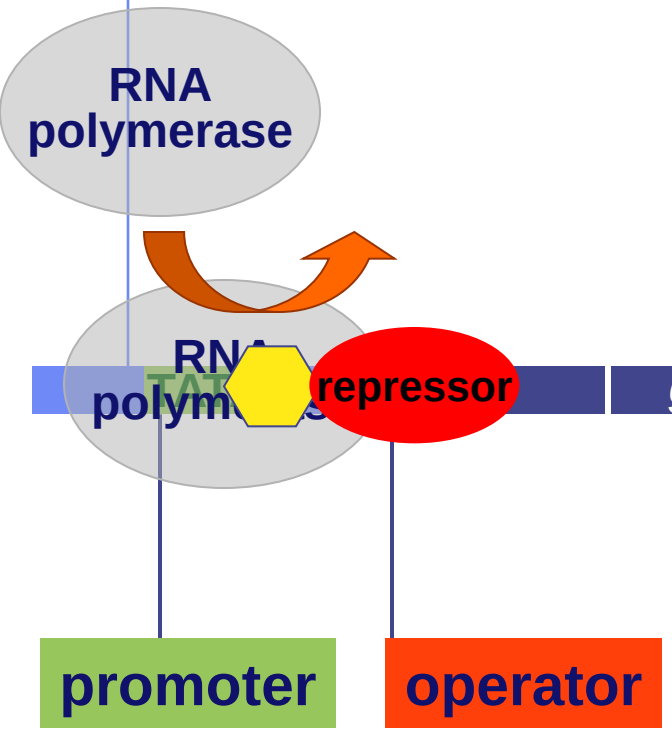
What happens when tryptophan is present?

Don't need to make tryptophan-building enzymes



Tryptophan binds allosterically to regulatory protein

Inducible operon: lactose



Digestive pathway model

When lactose is present, binds to *lac* repressor protein & triggers repressor to release DNA

- induces transcription

repressor — repressor protein

lactose

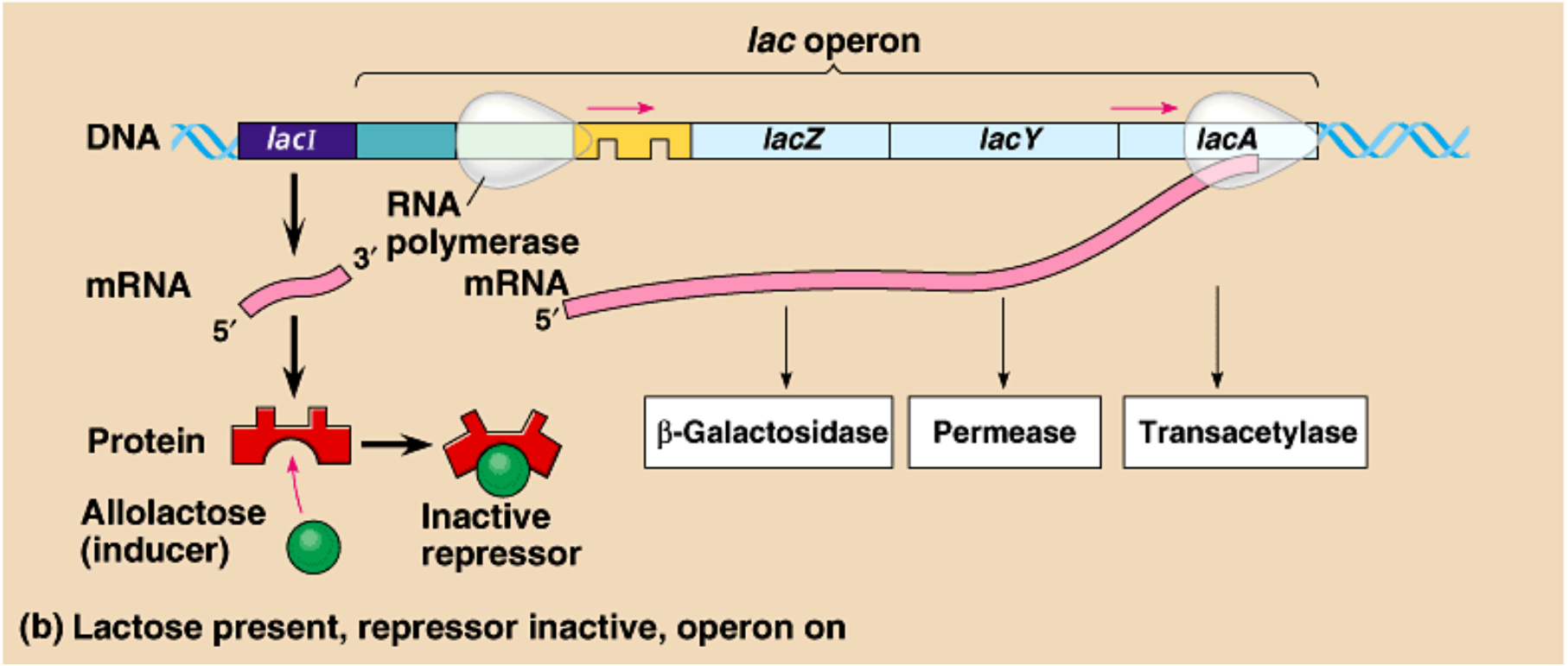
repressor — lactose – repressor protein complex

conformational change in repressor protein!

Lactose operon

What happens when lactose is present?

Need to make lactose-digesting enzymes



Lactose binds allosterically to regulatory protein

http://www.dnatube.com/video/22/How-lactose-turns-on-the-lac-operon

Jacob & Monod: *lac* Operon

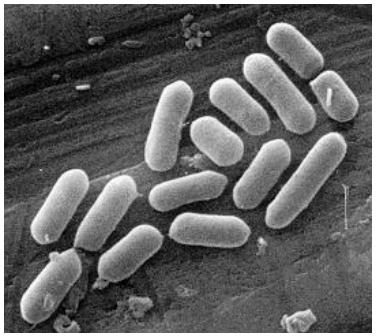
1961 | 1965

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

Jacques Monod

Francois Jacob

Operon summary

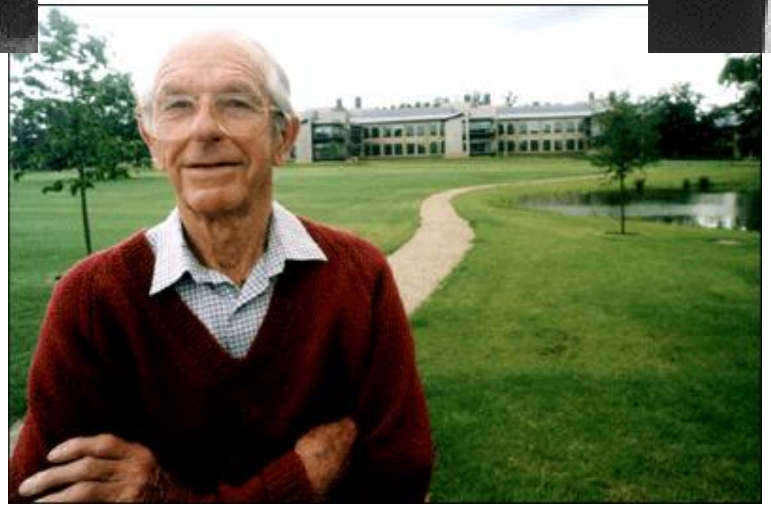


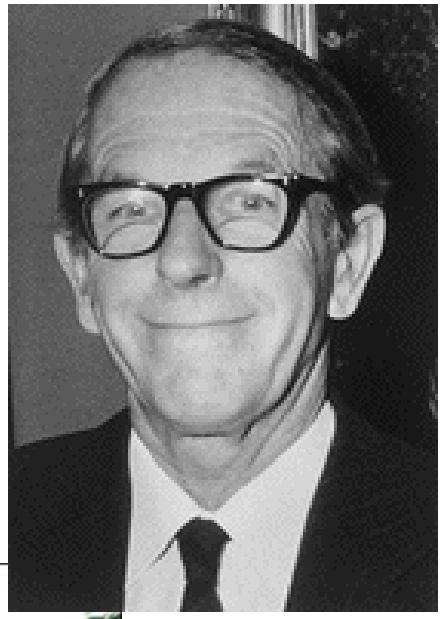
- **Repressible operon**
  - usually functions in **anabolic** pathways
    - **synthesizing** end products
  - when end product is present in excess, cell allocates resources to other uses
- **Inducible operon**
  - usually functions in **catabolic** pathways,
    - **digesting** nutrients to simpler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

Any Questions??

Fred Sanger

1958





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