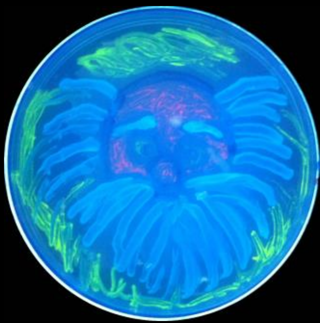
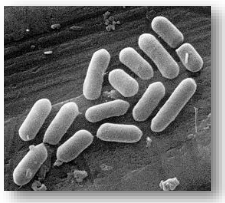


Biotechnology:
Bacterial Genetics

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

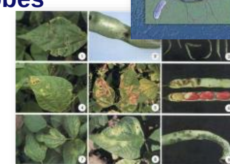
Bacteria



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

Bacteria as Pathogens

- Disease-causing microbes
  - plant diseases
    - wilts, fruit rot, blights
  - animal diseases
    - tooth decay, ulcers
    - anthrax, botulism
    - plague, leprosy, “flesh-eating” disease
    - STDs: gonorrhea, chlamydia
    - typhoid, cholera
    - TB, pneumonia
    - lyme disease



Bacteria as Beneficial (& necessary)

- Life on Earth is dependent on bacteria
  - decomposers
    - recycling of nutrients from dead to living
  - nitrogen fixation
    - only organisms that can fix N from atmosphere
      - needed for synthesis of proteins & nucleic acids
      - plant root nodules
  - help in digestion (E. coli)
    - digest cellulose for herbivores
      - cellulase enzyme
    - produce vitamins K & B_{12} for humans
  - produce foods & medicines
    - from yogurt to insulin

Bacterial Diversity

Borrelia burgdorferi
Lyme disease

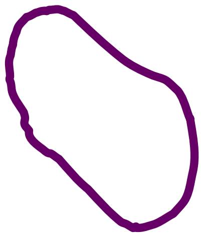
Treponema pallidum
Syphillis

Escherichia coli O157:H7
Hemorrhagic E. coli

Enterococcus faecium
skin infections

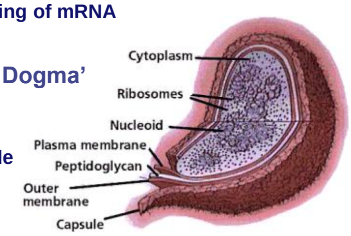
Bacterial Genome

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



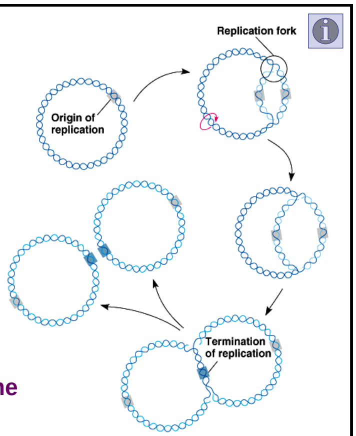
No Nucleus!

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



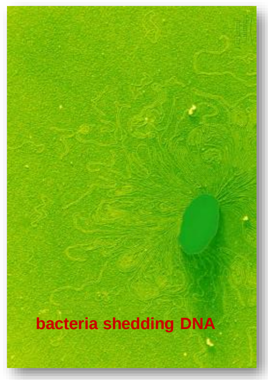
Binary Fission

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

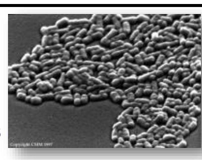


Variation in Bacteria

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

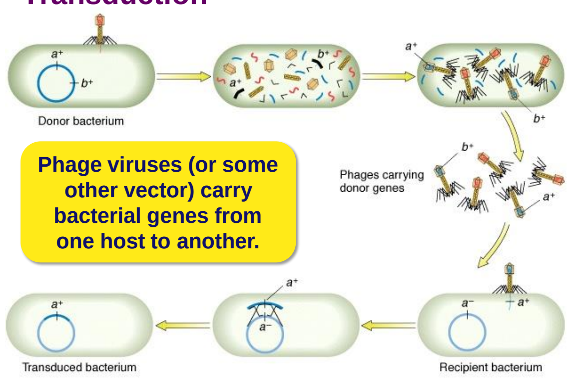


Spontaneous Mutation



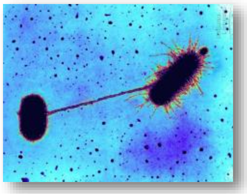
- Spontaneous mutation is a significant source of variation in <u>rapidly reproducing</u> species
- Example: *E. coli*
  - human colon (large intestines)
    - can reproduce every ~20 minutes
  - spontaneous mutations
    - for 1 gene, only ~1 mutation in 10 million replications
    - each day, ~2,000 bacteria develop mutation in that gene (out of the 2 x 10^{11} "new" bacterial cells)
    - but consider all 4300 genes, then:
      4300 x 2000 = 9 million cells with mutations per day per human host!

Transduction



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

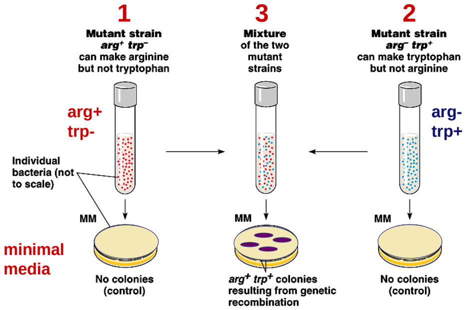


Transformation

- some 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

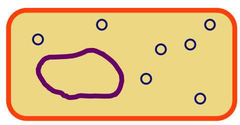
Swapping DNA

- Genetic recombination by trading DNA



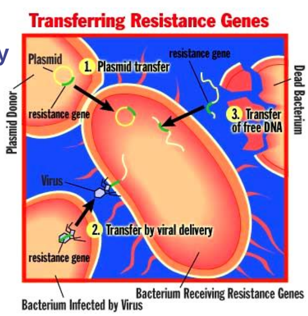
Plasmids

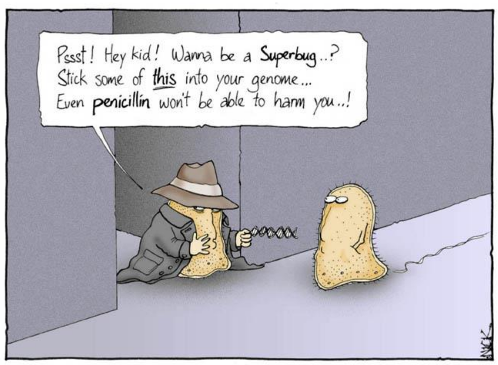
- Plasmids
  - small supplemental circles of DNA
    - 5000 - 20,000 base pairs
    - self-replicating
  - carry extra genes
    - 2-30 genes
  - can be exchanged between bacteria
    - rapid evolution
    - antibiotic resistance
  - can be imported from environment



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

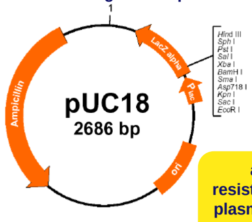




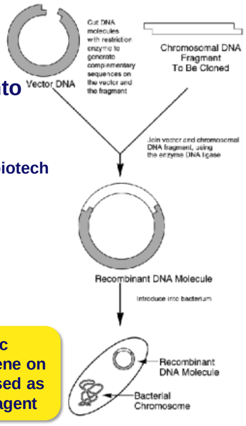
It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

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



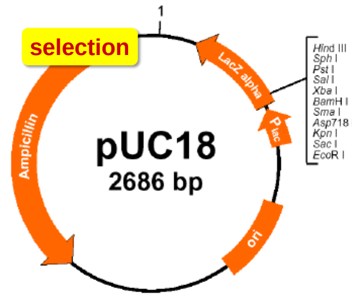
Recombinant Plasmid

- Antibiotic resistance genes as a selectable marker
- Restriction sites for splicing in gene of interest

Selectable marker

- Plasmid has both "added" gene & antibiotic resistance gene
- If bacteria don't pick up plasmid then die on antibiotic plates
- If bacteria pick up plasmid then survive on antibiotic plates
- selecting for successful transformation

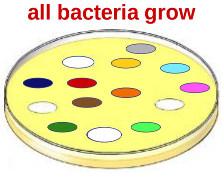
selection



Selection for Plasmid Uptake

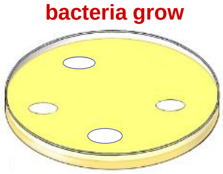
- Ampicillin becomes a selecting agent
  - only bacteria with the plasmid will grow on amp plate

all bacteria grow



LB plate

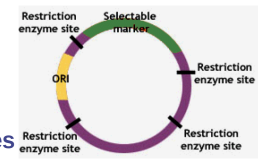
only transformed bacteria grow



LB/amp plate

Copy DNA

- Plasmids
  - small, self-replicating circular DNA molecules
    - insert DNA sequence into plasmid
      - vector = "vehicle" into organism
  - transformation
    - insert recombinant plasmid into bacteria
      - bacteria make lots of copies of plasmid
    - grow recombinant bacteria on agar plate
      - clone of cells = lots of bacteria
    - production of many copies of inserted gene



DNA → RNA → protein → trait

Development of GFP

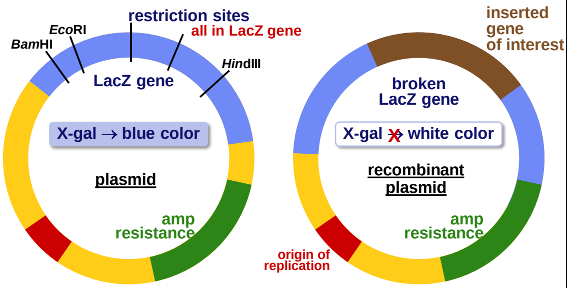
1961, 1994 | 2008

- Shimomura, Chalfie, Tsien
  - discovery, isolation, and purification of GFP and many fluorescent analogs

Osamu Shimomura Martin Chalfie Roger Tsien

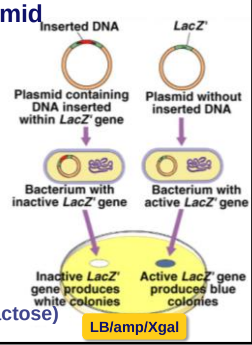
Need to Screen…

- Need to make sure bacteria have recombinant plasmid



LacZ is one Screening System

- Make sure inserted plasmid is desired recombinant plasmid
  - LacZ gene on plasmid produces digestive enzyme
    - X-gal → blue
    - blue colonies
  - insert foreign DNA into LacZ gene breaks gene
    - X-gal ✗ blue
    - white colonies
  - white bacterial colonies have recombinant plasmid when grown on a medium containing X-gal (analog of lactose)



Amp Selection & LacZ Screening