Why study bacterial genetics?
- It's 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

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
  - Syphilis
- 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 rapidly reproducing species
- Example: *E. coli*
  - human colon (large intestines)
  - 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!

**Transduction**
- Phage viruses (or some other vector) 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 pilii, attaches to female bacterium
  - cytoplasmic bridge allows transfer of DNA

**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**
- 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

**Transferring Resistance Genes**
- 1. Plasmid vectors
- 2. Plasmid by viral delivery
- 3. Chromosomal transfer

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

**pUC18**
- 2666 bp
- antibiotic resistance gene on plasmid is used as a selective agent

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**

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

- all bacteria grow
- only transformed bacteria grow

**LB plate**
- **LB/amp plate**

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

Need to Screen...
- Need to make sure bacteria have recombinant plasmid
**Amp Selection & LacZ Screening**

- **gene of interest**
- **LacZ gene**
- **amp resistance**

- Ampicillin in media
- LB/amp
- LB/amp/Xgal (lac)

**Application of Recombinant DNA**

- Combining sequences of DNA from 2 different sources into 1 DNA molecule
  - Often from different species
    - Human insulin gene in E. coli (Humulin)
    - Frost resistant gene from Arctic fish in strawberries
    - “Roundup-ready” bacterial gene in soybeans
    - BT bacterial gene in corn
    - Jellyfish glow gene in Zebra “Glofish” – GFP

**Development of GFP** 1961, 1994 | 2008

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

**Bacterial Genetics**

- Regulation of Gene Expression

**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

- **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

**Repressor Protein Model**

**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)

**Operon:**

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

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

What happens when tryptophan is present? Don’t need to make tryptophan-building enzymes!

DNA

mRNA

Protein

Tryptophan

Active repressor

DNA

mRNA

Protein

(m) Tryptophan present, repressor active, operon off

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)

RNA polymerase

DNA

gene1

gene2

gene3

gene4

Lactose

Lactose binds allosterically to regulatory protein.

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

Lactose Operon

What happens when lactose is present? Need to make lactose-digesting enzymes!

DNA

mRNA

Protein

β-Galactosidase

Peroxidase

Transacylase

Alloactose (inducer)

Inactive repressor

(b) Lactose present, repressor inactive, operon on

Lactose binds allosterically to regulatory protein.

Jacob & Monod: lac Operon 1961 | 1965

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

Any Questions??