The Stuff Microbial Life is Made of
Microbial Genetics

Terminology

- Genetics- Study of what genes are, how they carry information, how information is expressed, and how genes are replicated.

The Levels of Structure and Function of the Genome

- Chromosome
  - A discrete cellular structure composed of a neatly packaged DNA molecule
- Eukaryotic chromosomes
  - Located in the nucleus; they vary in number from a few to hundreds; they can occur in pairs (diploid) or singles (haploid); and they have a linear appearance
- Bacterial chromosomes:
  - Usually single, circular (double-stranded) chromosome, although many bacteria have multiple, circular chromosomes and some have linear chromosomes

- Genes: basic informational packets
  - Classical genetics: a functional unit of heredity
  - Molecular and biochemical genetics: site on the chromosome that provides information for a certain cell function
  - Preferred definition: a segment of DNA that contains the necessary code to make a protein or an RNA

- Genome- All of the genetic material in a cell
- Genomics- Molecular study of genomes
- Genotype- Genes of an organism
- Phenotype- Expression of the genes

The Size and Packaging of Genomes

- Variation in genome size
  - *Escherichia coli*: single chromosome with 4000 genes
    - 1-mm long if unwound and stretched linearly
    - 1000 times longer than the cell
- Human cell: 23,000 genes on 46 chromosomes

Central Dogma of Biology

- DNA =>RNA=>Protein
  - Replication
  - Transcription
  - Translation

Structure

- Nucleotide
  - Phosphate

  - Deoxyribose sugar
  - Nitrogenous base
- Double stranded helix
  - Antiparallel arrangement

Nitrogenous bases

- Purines
  - Adenine
  - Guanine
- Pyrimidines
  - Thymine
  - Cytosine

DNA

- Polymer of nucleotides: dATP, dTTP, dCTP, dGTP
- Double helix associated with proteins
- "Backbone" is deoxyribose-phosphate
- Strands held together by hydrogen bonds between AT and CG
- DNA is copied by DNA polymerase
  - Synthesis of the new strand is in the $5' \rightarrow 3'$ direction
  - Initiated by an RNA primer
  - Leading strand synthesized continuously
  - Lagging strand synthesized discontinuously
    - Okazaki fragments
    - RNA primers are removed and Okazaki fragments joined by a DNA polymerase and DNA ligase

- DNA replication is semiconservative

Leading strand

- RNA primer initiates the 5' to 3' synthesis of DNA in continuous manner

Lagging strand

- Multiple Okazaki fragments are synthesized
- Okazaki fragments are ligated together to form one continuous strand

Transcription

- DNA is transcribed to make RNA (mRNA, tRNA, and rRNA)
- mRNA- RNA that carries information from DNA to the ribosome sites of protein synthesis in the cell.
  - Once mRNA has been transcribed from DNA, it is bound to ribosomes and translated into protein.
  - After a certain amount of time the message degrades into its component nucleotides, usually with the assistance of RNases.

- Copy of a structural gene or genes of DNA
  - Can encode for multiple proteins on one message
- Thymidine is replaced by uracil
- The message contains a codon (three bases)

- tRNA- small RNA chain of about 74-93 nucleotides that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation.
  - It has sites for amino-acid attachment and an anticodon region for codon recognition that binds to a specific sequence on the messenger RNA chain. It is a type of non-coding RNA.

- Copy of specific regions of DNA
- Complimentary sequences form hairpin loops
  - Amino acid attachment site
  - Anticodon
- Participates in translation (protein synthesis)

- rRNA- Ribosomal RNA (rRNA) is the primary constituent of ribosomes.
  - Ribosomes are the protein-manufacturing organelles of cells and exist in the cytoplasm.

- Consist of two subunits (70S)
- A subunit is composed of rRNA and protein
- Participates in translation

- Transcription begins when RNA polymerase binds to the promoter sequence
- Transcription proceeds in the $5' \rightarrow 3'$ direction
- Transcription stops when it reaches the terminator sequence

Codons

- Triplet code that specifies a given amino acid
- Multiple codes for one amino acid
- 20 amino acids
- Start codon
- Stop codons

Translation

- mRNA is translated in codons.
- Translation of mRNA begins at the start codon: AUG
- Translation ends at a STOP codon: UAA, UAG, UGA
- tRNA anticodon with attached amino acid binds to the start codon
- Ribosomes move to the next codon, allowing a new tRNA to bind and add another amino acid

Regulation of Bacterial Gene Expression

- Constitutive enzymes are expressed at a fixed rate
- Other enzymes are expressed only as needed
  - Repressible enzymes
  - Inducible enzymes

Mutations

- Changes made to the DNA
  - Spontaneous – random change
  - Induced – chemical, radiation.
  - Point – change a single base
  - Nonsense – change a normal codon into a stop codon
  - Back-mutation – mutation is reversed
  - Frameshift – reading frame of the mRNA changes

Effects of mutations

- Positive effects for the cell
  - Allow cells to adapt
- Negative effects for the cell
  - Loss of function
  - Cells cannot survive

- Base substitution (point mutation)
  - Missense mutation
    - Change in one base
    - Result in change in amino acid

- Nonsense mutation
  - Results in a nonsense codon

- Frameshift mutation
  - Insertion or deletion of one or more nucleotide pairs

- Ionizing radiation (X rays and gamma rays) causes the formation of ions that can react with nucleotides and the deoxyribose-phosphate backbone.
- Nucleotide excision can repair mutations.

- UV radiation causes thymine dimers
- Light-repair separates thymine dimers

The Frequency of Mutation

- Spontaneous mutation rate = 1 in 109 replicated base pairs or 1 in 106 replicated genes
- Mutagens increase to 10–5 or 10–3 per replicated gene

Selection

- Positive (direct) selection detects mutant cells because they grow or appear different.

- Negative (indirect) selection detects mutant cells because they do not grow.

Genetic Transfer and Recombination

- Vertical gene transfer
  - Occurs during reproduction, between generations of cells
- Transfer of genes between cells of the same generation
  - Horizontal gene transfer

Transformation

- Nonspecific acceptance of free DNA by the cell (ex. DNA fragments, plasmids)
- DNA can be inserted into the chromosome
- Competent cells readily accept DNA

DNA released from a killed cell can be accepted by a live competent cell, expressing a new phenotype.

Genetic Recombination

- Exchange of genes between two DNA molecules
  - Crossing over occurs when two chromosomes break and rejoin

Transduction

- Bacteriophage infect host cells
- Serve as the carrier of DNA from a donor cell to a recipient cell
  - Generalized
  - Specialized

Genetic transfer based on generalized transduction.
Genetic transfer based on specialized transduction.

Plasmids

- Conjugative plasmid- Carries genes for sex pili and transfer of the plasmid
- Dissimilation plasmids- Encode enzymes for catabolism of unusual compounds
- R factors- Encode antibiotic resistance

Transposon

- “Jumping genes”
- Exist in plasmids and chromosomes
- Contains genes that encode for enzymes that remove and reintegrate the transposon
- Small transposons are called insertion elements

Movement of transposons can occur in plasmids and chromosomes.