Microbial Genetics: Chapter 8

Genetics: science of heredity; includes study of genes (carry information, replication, expression)

*Chromosomes made of DNA contain organism's entire genome: double stranded in most cells.
*Genes: segments of DNA—code for proteins
*Composition:
Macromolecule of repeating units of nucleotides
Nitrogen base: purines; adenine, guanine
pyrimidines: thymine, cytosine, uracil (RNA)
Pentose sugar: deoxyribose or ribose
Phosphate [Nucleosides are base and pentose (no phosphate)]
Sugar-phosphate backbone with base attached to each sugar
Twisted together in double helix
Linear sequence of bases provides actual information: genetic code determines how sequence converted to amino acids sequence of proteins
Base sequence of one strand determines the sequence of other strand due to pairing of bases: adenine---thymine
cytosine---guanine
This complementary structure allows for precise replication
Paired DNA oriented in opposite directions (anti parallel):
End with hydroxyl attached to 3'C is 3'end
End with phosphate attaced to 5'C is 5' end
5'→3' one strand/ 3'→5' other strand

Genotype (potential properties) and Phenotype (expressed properties-gene expressed as protein)

Bacteria: single, double stranded, circular chromosome; looped, folded, and attached to plasma membrane/supercoiled by topoisomerase II or **DNA gyrase** (antibiotic ciprofloxacin (a fluoroquinolone) inhibits gyrase activity

REPLICATION: one double stranded DNA to 2 daughter molecules

1. Parent DNA unwound (by **helicase**) and stabilized (small DNA segment after another) origin of replication –replication site at fixed point
2. Free nucleotides (in cytoplasm) matched up to exposed bases
   T—A
   C—G
3. Once aligned the newly added nucleotide joined to growing DNA by **DNA polymerase** (only adds new nucleotides to 3' end/ 5' →3' direction)
   Leading strand synthesized continuously moving toward replication fork
   Lagging strand synthesized discontinuously since can only add to 3' end
   RNA polymerase synthesizes a short piece of RNA (RNA primer) needed to begin addition of nucleotides which are then added toward 3' ends in short fragments--Okazaki fragments

Moves away from replication fork
DNA polymerase removes RNA primer as bases added
DNA ligase joins Okazaki fragments
DNA polymerase generally corrects errors during replication (proofreading)
so error rate = 1/billion nucleotides

4. Original strand and new strand rewound/ each has one original(conserved) strand and one new strand (semiconservative replication)
When nucleoside triphosphate bonds to sugar—release of two phosphates that provide energy for reaction

TRANSCRIPTION: Synthesis of complementary strand of RNA from DNA template

Types of RNA: messenger (mRNA)
ribosomal (rRNA)
transfer (tRNA)

Sequence of complementary bases assembled from free nucleotides by action of **RNA polymerase**

guanine ---cytosine; adenine----uracil
ATGCAT→mRNA of UACGUA

1. RNA polymerase binds to DNA template at promoter site (only one strand of DNA used)
2. New RNA chain grows in the 5' to 3' direction
3. RNA synthesis continues until RNA polymerase reached terminator site
4. New strand of RNA released from DNA template

Transcription allows cell to create short term copies of genes for specific protein synthesis

TRANSLATION: decoding mRNA to make protein using codons of three nucleotides for each amino acid

Degeneracy of code (64 possible codons/20 amino acids)
61 codons for amino acids
3 nonsense codons or stop codons (UAA, UAG, UCA)
1 start codon (AUG)/also for amino acid formylmethionine

**2 subunits of Ribosome for orderly binding of tRNA and assembly of amino acids into protein

**tRNA has anticodon complementary to mRNA codon and carries specific amino acid

1. On ribosome, mRNA set up initiator AUG to start translation; tRNA with anticodon UAC
2. The second codon of mRNA pairs with a tRNA carrying the second amino acid/translated in 5' to 3' direction
3. Ribosome joins two amino acids with peptide bond (first tRNA leaves ribosome) and ribosome moves along mRNA forming a polypeptide chain
4. Translation ends when one of the three nonsense codons is reached
5. Ribosome separates into two subunits, mRNA and new polypeptide chain released

Ribosome, mRNA, and tRNA available to be used again

Prokaryotic cells: Transcription and Translation can occur at same time WHY??
Eukaryotic cells: Transcription completed first and has RNA processing Why??

Is it important to regulate protein synthesis/gene expression?
Three types of regulation:
- Enzyme inhibition (feedback inhibition)
- Repression (tryptophan operon)
- Induction (lactose operon)

60-80% genes Constitutive (always turned on)
Operon: collection of closely associated genes next to each other on chromosome and regulated together (1961-Francois Jacob and Jacques Monod)
Figure 8.14 in text

Mutations: change in base sequence of DNA
- Do they always change the genetic code? (silent mutation)

Types of mutation:
- *Base-pair mutation:
  - --Missense mutation
  - --Nonsense mutation
- *Frameshift mutation
  - --changes the reading frame

Causes of mutations:
- *Chemicals (nitrous acid)
  - Nucleoside analog
  - Frameshifts due to deletions or insertions
- *Physical (UV light)
- *Biological mutagens (transposons)

Frequency of Mutations:
- Spontaneous mistakes in base replication about 10^{-9}
- Since about 10^3 base pairs/gene: about 1 in 1,000,000 genes

Bacteria used to study mutations:
- Only have one chromosome…one copy of each gene
- Easy to grow

Direct Selection of Mutation:
- Colony color
- Motility
- Antibiotic resistance

Indirect Selection of Mutation:
- Way to look at traits that are not easily identified, at changes in metabolic pathways
- Replica plating:
  - Auxotrophs from Prototrophs

Test chemicals for mutagenicity…..Ames test