BIOL 240 *GENERAL MICROBIOLOGY* (Part II) – Important Terms and Concepts 10/11/2009

5. METABOLISM, enzymes, cofactors, regulating enzyme activity (pH, temperature, [substrate]); coenzymes, **feedback inhibition**, reaction rate. Collision theory, Apoenzyme, Holoenzyme. **Denaturation**, **Competitive & Noncompetitive Inhibition**. Ribozymes, Enzymes, cofactors, **regulating enzyme activity**; coenzymes, feedback inhibition, reaction rate, Equilibrium, ***Activation Energy***, saturation. **Redox reactions**; Reduction, Oxidation; Dehydrogenation; **ATP.** Light & Chlorophyll; **NADH**. ***Substrate-level Phosphorylation***. Carbohydrate catabolism: Glycolysis (invest 2 ATP, harvest 4 = 2 net; 6C glucose → **2X** (3C Pyr + 2NADH), **2X** Pyruvate Oxidation (3C→ 2C Acetyl-CoA + NADH + CO_2); **2X** Krebs/TCA cycle (2C + 4C → 6C→5C + CO_2 + NADH→ 4C + CO_2 + NADH → ATP → $FADH_2$ → NADH; = **6 NADH, 2$FADH_2$, 4CO2, 2 ATP per glucose in TCA cycle**), Electron Transport Chain (ETC; **Proton Motive force**, **Chemiosmosis**, Ubiquinone/ coenzyme Q, Cytochrome C, Cytochrome C Oxidase, **O_2**; ***ATP Synthase***). **2ATP/$FADH_2$, 3ATP/NADH**. Prokaryotes harvest **38 ATP/glc,** Eukaryotes harvest **36 ATP/glc**.

- **Anaerobic Respiration**: Nitrate, Sulfate, or Carbonate electron acceptors – none yields as much energy as O_2. **Fermentation** = only **2 net ATP/glc;** organic electron acceptor with lots of energy left over – regenerates NAD+ for glycolysis.
- **Photosynthesis**: **Light reactions** harvest light as High Energy Electrons → ATP and ***NADPH*** → used to add CO_2 carbons to 5C RuBP → glucose/sucrose/starch!!! (**Carbon Fixation** in the **Calvin-Benson Cycle**/ **Dark Reactions**!!). Chemoheterotrophs, Chemoautotrophs, Photoautotrophs, Photoheterotrophs. ***Anabolic and catabolic pathways are tied together ("coupled" -- feed off of each other for Carbon and Energy)***!!

6. MICROBIAL GROWTH: Physical Requirements (Temp = psychro-, meso-, thermo-philes; pH = acido-, neutero-, alkalo-philes; Osmotic pressure = halophiles/ "salt-lovers"); Chemical Requirements C, N, S, P & Trace elements; O_2 (toxic = Singlet, Superoxide, Peroxide anion, Hydroxyl radical): ***obligate aerobes, facultative aerobes, obligate an-aerobes, aerotolerant anaerobes, microaerophiles***. – **Superoxide Dismutase, Catalase, Peroxidase**. Culture Media: Chemically Defined, Differential, Selective. **Binary Fission**. **Bacterial growth curve (lag, log, stationary, death phases).** Measuring growth: Plate counts, MPN test, Turbidity w/ spectrophotometer.

7. GROWTH CONTROL: Sepsis, Asepsis, Sterilization, Disinfection v. Antisepsis, **Bacteriostatic v. Bacteriocidal**. – Death at logarithmic rate! Depends on organic matter, pH, Temp, biofilms).

- Physical Methods: Heat (TDP, TDT) – Moist heat - ***Pasteurization***, ***Autoclaving***. Also: Filtration, low Temp, High Pressure, Desiccation, Osmotic Pressure, Readiation (ionizing – free radicals).
- Chemical Methods: Use-dilution test, Disk-diffusion method. TYPES (13): **Phenol / phenolics / bisphenols** (triclosan!) = denature proteins, dissolve membranes, Biguanides = disrupt membranes, **Halogens** = oxidizers (damage DNA, proteins, membranes), **Alcohols** = denature proteins, dissolve lipids, **Heavy metals** = *oligodynamic action*/ denature proteins, **Surfactants** = detergents & Quats = denature prots., disrupt memb., Food Preservatives = Organic acids, Nitrite, antibiotics – inhibit metab.; Aldehydes = crosslink, Gases = denature, Peroxygens = oxidize. ***Few Disinfectants effective on endospores or mycobacteria!!***

8. (a.) MICROBIAL GENETICS: Genetics, Gene, Genome, Genotype, Phenotype. E. coli as Model Organism. ***Central Dogma***: **DNA (replicates) → (transcription) RNA → (translation) Proteins**; DNA: A=T, G≡C. ***Semi-Conservative & Bidirectional*** **Replication**, Antiparallel Strands, **Origin of Replication**, **5'→3' synthesis**, **DNA Polymerase III**, **RNA Primase**, RNA Primers, Single-Strand Binding Proteins (SSB), template strands, **DNA Polymerase I**, **DNA Ligase.** Continuous leading strand, discontinuous Lagging Strand Synthesis.

- **Transcription: RNA Polymerase; mRNA, tRNA**, rRNA, 5'→3' from **PROMOTER** on DNA template, unidirectional synthesis!
- **Codons/Genetic Code** – **stop (UAA, UAG, UGA) and start (AUG) codons**.

FOR MIDTERM 3 (preview):

- **Translation**: mRNA + tRNA with Amino Acid attached (and AntiCodon) + Small Ribosomal subunit + Large Ribosomal subunit. **Ribosome** moves 5'→3' along mRNA template, transfers growing strand in **P-Site** to new Amino acid on tRNA in **A-site** (Peptidyl Transferase); Peptide synthesized **Amino (N) Terminus → Carboxyl (C) Terminus** according to mRNA codons starting with AUG/methionine. Constitutive Enzymes, Repressible Enzymes, Inducible Enzymes.
- ***OPERONS***: = ***promoter + operator + structural genes, regulated by a Repressor protein.*** **Lac Operon (catabolic)** = ON if Lactose present (repressor OFF when BOUND by lact. signal) AND glucose absent; **Trp Operon (anabolic/ biosynthetic)** = ON if Trp end product NOT in excess and bound to Trp Repressor (repressor OFF when UNBOUND by Trp signal).
- **Horizontal Gene Transfer:** Genetic Recombination – Vertical & Horizontal transfer. **Transformation** (Griffiths: R→S *pneumococcus*), recombination/crossover into chromosome ("homologous gene replacement"), **Conjugation** (**F factor**/ ***Plasmid***, sex pilus, Hfr Cells; F+ donor cells, F- recipient cells). Bacteriophage **Transduction**. R-Factors (MDR), Transposons.
- Mutations: chemical and radiation ***mutagens***. ***DNA Excision Repair*** (Endonuclease, DNA Polymerase I, Ligase). **Missense, Nonsense, Frameshift mutations.** (& "Silent" or Synonymous mutations). Positive/Direct and Negative/Indirect Selection, Replica Plating. **AMES TEST** for Mutagens/Carcinogens.

Microbiology Midterm 2 (Fall 2009): Study Questions

Possible Short Essay Topics (be prepared to draw diagrams as well!):

1. **Diagram and describe how enzymes speed up chemical reactions, and how they affect the energy and equilibrium of a reaction. Describe *6* different physical and chemical factors that can regulate enzyme activity.**
2. Diagram and describe an example of *energetic coupling* in a cell. Define energetic coupling.

3. **Describe briefly or diagram how six carbons in glucose are all transferred and released, and in what form, from glycolysis through the Krebs (TCA) cycle.**
4. **Compare/contrast the energy inputs and outputs of aerobic respiration vs. fermentation. Include all phosphorylated compounds and high energy electron carriers.**
5. **How is the energy from high energy electron carriers converted to ATP in mitochondria and aerobic bacteria? Be sure to include the proper terms for each of at least 2 processes/ theories involved in energy conversions, and two of the proteins/enzymes involved in the final steps of making ATP.**
   [hint: discuss electrons, gradients, and phosphates, and the terms for the pathways involved]

6. Draw a simple diagram showing how energy is captured from light in Photosynthesis, and then how this energy is used to capture carbons from atmospheric CO_2 during carbon-fixation in the Calvin-Benson cycle.

7. **Distinguish the carbon and energy sources for all of the trophisms: chemo-, photo-, hetero-, auto-, and combinations of each.**
8. List and describe the three types each of pH and temperature preferences for growth among diverse bacteria.
9. Describe how oxygen can be both a required part of metabolism, and a toxin to the same microorganism. How do aerobes overcome this toxicity? Draw diagrams of the five different oxygen-related growth phenotypes in an agar deep tube.

10. **Diagram and thoroughly define the four phases of a typical bacterial growth curve. What is happening to the cells during each phase?**
11. Distinguish between Sterilization, Disinfection, and Antisepsis, and give an example of a physical or chemical method to accomplish each. Also, distinguish between germistatic activity, and germicidal activity of an antimicrobial agent.

12. **Explain how the Central Dogma of molecular genetics illustrates how hereditary Genotype becomes hereditary Phenotype in an organism (3 processes involved in Heredity and "Gene Expression").**
13. **Describe 6 properties of DNA structure and DNA replication that are essential to its successful synthesis, structure, and function.**
14. **Using a diagram, compare and contrast DNA replication at the leading and lagging strands of a replication fork. Include at least 6 protein and RNA molecules involved.**
15. Distinguish between the starting sequences and ending sequences and enzymes used to initiate, polymerize, and terminate Replication and Transcription.

Preview for Midterm 3:

16. Distinguish between the starting sequences and ending sequences and enzymes used to initiate, polymerize, and terminate Replication, Transcription, and Translation.
17. Diagram the structure of an Operon, and label and define the function of at least 5 protein and DNA components involved in its function and regulation.

****** NOTE: One ESSAY Question may be MANDATORY on this exam!!!***

- ***[**Bold-typed questions address especially important concepts for Part II of the course!]***
- ***TIP: For "comparison/contrast" or "distinguish" questions, use a table with explanations.***