BIOL 230 (Part III) – Major Terms & Concepts (this is NOT an exhaustive list!!) 11/11/2018

Sadava, et al., 2016 (11e); Chapter Number:

13. DNA REPLICATION: (*REVIEW:*) semiconservative, dispersive, conservative; **DNA Repair:** Proofreading, mismatch repair, excision repair – "seeker" proteins, endonucleases, Dpol1, Ligase, Deoxyribonucleoside triphosphates.

- **Meselson/Stahl** – CsCl density gradient; **^{14}N, ^{15}N**; intermediate DNA density = semiconservative.
- **Kornberg**, *DNA Polymerase*; Deoxynucleoside triphosphates, pyrophosphate, DNA template 3'-Hydroxyl terminus, 5'-Phosphate terminus
- **Initiation, Elongation, Termination: DnaA, DNA polymerase III**; Origin of replication (***Ori, Ter***), Helicase, ATP, ssDNA-binding proteins, **Primase** (RNA Primer, free 3'-OH), DNA Polymerase **I**, DNA Ligase, **5'→3'** synthesis; *DNA Gyrase/Topoisomerase* **Leading strand, lagging strand, Okazaki fragments**; **DNA polymerase I, DNA Ligase**

DNA REPAIR: Proofreading, mismatch repair, excision repair – ***"seeker" proteins***, repair endonucleases, Dpol1, Ligase.

***TELOMERES;* Telomeres**, Telomerase, ….(GGAATT)n…… Protein-RNA enzyme with its own template for synthesis;

DNA sequencing, dideoxy Nucleotide triphosphates ("chain-terminators", ddNTPs).

Polymerase Chain Reaction (PCR) – denature, anneal ***DNA Primers (specific!)***, elongate; ***Taq* DNA Polymerase. =** *In Vitro* molecular cloning.

14. Beadle & Tatum – ***One-Gene-One Polypeptide***; auxotrophic mutants; Arg-; biochemical pathways = genetic pathways!

- ***Central Dogma (Crick!)***; messenger, adapter
- ***Gene expression***. DNA → RNA → Protein.

TRANSCRIPTION **Promoter**, RNA polymerase; Ribonucleoside triphosphates; Eukaryotes (PROCESSING: -- **Introns, Exons, RNA Splicing – snRNPs; 5'-GTP "Cap", 3'-polyA "Tail".** Codons: triplet "words", nonoverlapping, degeneracy, "Wobble", **Start:** 5'-AUG-3', **Stop:** UAG, UGA, UAA.

TRANSLATION, tRNA, methionine, AUG; Reading Frame; **Ribosome** – large (peptidyl transferase) and small subunits, Ribozyme; Amino (N) terminus, Carboxyl (C)- terminus; **Aminoacyl tRNA synthetases** (activating enzyme); AMP, "charged" tRNA; Translation initiation complex; **N→C** synthesis; A-site, P-site; **Release Factor**; Posttranslational Regulation – delivery signals; Antibiotic regulation

Point mutations (Ch. 15) – **silent / synonymous, missense, nonsense, frameshift**; Chromosomal mutations – deletion, inversion, reciprocal translocation; Spontaneous Mutations, Induced Mutations.

16. Viral, Prokaryotic & Eukaryotic Gene Regulation

Lytic vs. Lysogenic Phage reproductive cycles – regulation (early genes, late genes, capsid); Cro Protein (↑lytic), cI Protein (↑lysogenic);

- *(Ch. 12, 15)* **Conjugation, Transformation, Transduction**; *(Ch. 17)* **Transposons**, transposase; Structural Genes ; R-Factors, F-factors/plasmids. *(Chs., 18, 12)*

Operon, Operator, Promoter, Inducer, **Repressor** Positive control, Negative control, ***i* Gene; *Inducible*** promoter, ***Constitutive*** promoter; cyclic-AMP, cAMP-repressor Protein (**CRP** or CAP)

Lac Operon; Inducer (lactose), ***Trp*** Operon.

- **Eukaryotic Genome & Gene Regulation:** Nuclear envelope, compartmentalization. Satellite DNA, ***telomeres, telomerase***. ….(GGAATT)n…

Levels of Control: ***Transcription, Post-Tsc/RNA Processing, Translation, Post-translation.***

Promoter, Transcription Factors, Enhancer, Silencer, Activator, Repressor; Coordinate Regulation; Alternate Splicing.

Primary transcript/ Pre-mRNA; ***Spliceosome'*** **Introns, Exons, RNA Splicing -- snRNPs**

5'-GTP "Cap", 3'-polyA "Tail".

Rpol I (rRNA), II (mRNA), III (tRNA and snRNAs).

Alternate splicing, mRNA stability; ***Heterochromatin***, Euchromatin; ***XIST* gene**; **X-inactivation**, Barr Body. **Signal Sequences**, Signal Recognition Particle. Polysomes, protein modification, **Ubiquitin**, **Proteasome**. [[& See Ch. 16 Review slides!!]].

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Chs. 15/17/18. Cloning Genes ddNTPs, Sanger DNA Sequencing; PCR, *Taq* DNA polymerase, specific synthetic DNA Primers. Recombinant DNA, **Restriction Endonucleases**; Restriction sites, "sticky ends", ligate/splice DNA; DNA gel electrophoresis, DNA denaturation, **Hybridization**, labeled Probes**.**

Vectors – plasmid, virus, antibiotic-resistance markers (selectable marker gene, polycloning site, disruptable reporter gene, *Origin* of replication (& circular for bacteria)

Cloning Genes: Clone = many copies of a piece of DNA, or making an identical individual organism or cell (naturally or artificially / *in vitro*).

Cloning vector – (*in vivo* cloning) ***replicon*** (*Ori*, telomeres?, centromeres? Circular vector?), Restriction site (PCS), Selective markers (antibiotic resistance, nutritional ability, etc.). **Gene Libraries**: ***cDNA***, ***genomic DNA***. ***Reverse Transcriptase***. Labeled cDNA probe, hybridization, **Gene Chips** (DNA Microarrays). **Expression vectors** (need Tscn start and stop, Ribosomal Binding Site for translation, etc.) to make gene product in recombinant host (such as bacteria or yeast).

Reporter Genes (reporter vectors) – visible gene product "reports" activity of any attached promoter!! GFP, *LacZ*, X-gal.

Recombinant DNA. Transgenic organisms.

CRISPR: **Cas9** endonuclease, PAM, **gRNA** (guide RNA), ***homology-directed repair*** replacement DNA sequence.

****** Be ready to transcribe and translate a DNA gene sequence!! ******

- ***ESSAYS Preparation note:*** *A good strategy for answering **comparison and contrast** questions is to make a **TABLE** with a column for each **category/topic** to be compared. Then compare related **characteristics** in the listed rows below each topic.*
- ***Remember: All questions are important study tools for the entire exam**, though the questions in **BOLD** are the most likely questions to be asked in essay form on the test. **Practice answering EVERY question THOROUGHLY and REPEATEDLY!!!***

BIOL 230: Cell & Molecular Biology -- Midterm 3 (Fall 2018): Study Questions

Possible Short Essay Topics *(be prepared to draw diagrams & give thorough explanations as well!)*:

1. *Ch. 13:* Diagram a replicating fork of DNA, and at least 7 protein and nucleic acid (RNA) molecules, in sequential order, that participate on each strand during synthesis. *Briefly explain the function of each molecule involved.* Indicate the proper name and direction of each replicating strand, and the type of synthesis associated with each.

2. ***Ch. 14:* Describe the three processes involved in Heredity and "Gene Expression", and the major enzymes and molecular polymers produced, that define the direction and flow of genetic information in living systems. What is the name of this fundamental Theory voiced by Sir Francis Crick? *How does this theory explain the production of phenotype from hereditary genotype?***
3. Diagram the 4 major components that initiate translation of a genetic message. Distinguish between the start and stop sites of transcription on a DNA template, and the start and stop sites of translation on an RNA transcript.
4. Describe and Diagram how a messenger RNA is read and encoded into the language of amino acids/ polypeptide from the nucleic acid code. Include the direction of reading a transcript, the direction of polypeptide synthesis, what sets the "frame" of reading the genetic code, and how translation is terminated.
5. **Distinguish between the starting sequences and ending sequences and enzymes used to initiate, polymerize (elongate), and terminate Replication, Transcription, and Translation. Define each process, including directions of synthesis, enzymes involved, nucleotide sequences recognized, and the type of molecule produced. Be sure to discuss main differences in numbers and directions of "bubbles" and "forks".**
6. Describe and illustrate the differences between four different types of point mutations in DNA. Which type(s) of mutations are likely to produce the most severe phenotype, and why? What factors determine the severity of the phenotype (physical changes) in an organism resulting from a point mutation?

7. ***Chs. 15/17/18:* Describe the processes of PCR, DNA sequencing, molecular cloning with hybridization, and gene-editing with CRISPR/*Cas9*. What important reagents/ingredients and procedures/treatments are necessary for each? Explain how most of these methods were derived from fairly basic knowledge of DNA structure and replication, and how they are used for modern biotechnological applications today.**
8. Describe the **4 main ingredients of a PCR reaction**, and diagram and describe the three steps in a cycle. How do these work to amplify (make many copies of) a SPECIFIC segment of DNA out of a whole genome?? How many PCR cycles would you need to amplify 1 DNA segment _____ (number to be given at exam) times? *(be prepared to do the math!)*
9. **Diagram the 4 essential components of a bacterial Cloning Vector (plasmid). How would you insert your DNA/gene of interest** (such as a human or other mammalian gene) **into this bacterial DNA carrier? How will you know that recipient bacteria have taken-up your vector? How will you know that vector taken up by bacteria contains an insertion of foreign DNA? How would you identify which recombinant vector has your particular gene/sequence of interest?**

10. ***Ch. 16a:* Diagram the lytic and lysogenic reproductive cycles of a bacteriophage. When might one replicative cycle be advantageous over the other? How is the switching between these two reproductive cycles genetically regulated (be sure to mention the regulatory proteins involved)?**
11. Using diagrams, compare and contrast the three different methods of **"horizontal" transmission of genetic information between bacterial cells**. *What must happen to the transferred DNA for it to be stably inherited and transmitted to later generations?*****
12. **Define and Diagram the structure of the *Lac* Operon** *(hint: not all components are unique to this operon)*, ***DEFINE what an operon is,*** **and label and define the function of at least 7 proteins, small molecules, and DNA components involved in operon function and in both positive and negative regulation. Describe how Inducible Operons are energetically efficient systems for a cell** *(what 2 conditions must be met for it to turn ON?)*.
13. **Compare and contrast regulation of the *LAC* Operon and the *TRP* Operon. *DEFINE what an operon is.* When is each turned ON or OFF? *Draw each operon in the PRESENCE of its own ligand (signal molecule).* What controls the activity of the regulatory proteins involved (both positive and negative regulation)? *Explain how each type of regulation is appropriate for an operon encoding catabolic or anabolic enzymes*** *[HINT: How does each contribute to greater efficiency for a cell?, by conservation of energy and materials for the cell??].*

14. *Ch. 16b:* Describe and Diagram the interactions between **6 protein and DNA factors involved in ONLY Eukaryotic gene regulation**. How does coordinate gene regulation differ between Prokaryotes and eukaryotes?
15. **Describe and DIAGRAM at least 8 ways that gene structure, transcription, and transcriptional and post-transcriptional regulation differ between Prokaryotes and Eukaryotes. What differences between prokaryotes and eukaryotes also exist in the translation of an mRNA transcript?**
16. **Describe and Diagram the 4 steps that may occur in Post-Transcriptional Processing of a eukaryotic primary transcript into a mature mRNA ready for translation. Describe how unique mechanisms in Eukaryotic gene regulation produce exceptions to Beadle and Tatum's Theory of the relationship between genes and proteins (state the Theory).**
17. **Compare the advantages and disadvantages of eukaryotic gene regulation at the Transcriptional level, versus regulation at the Post-Translational level. Cite specific examples of each type of regulation.**