MIDTERM 4 LEARNING OBJECTIVES

1. **Compare and contrast DNA and RNA in terms of: a. structure b. function c. common state within a cell d. synthesis (features of the polymerase and requirements)**
    a. Structure: deoxyribose and ribose both have 1' OH, deoxy has 2' H, and then A/T/U/G/C is attached to 5'
        i. RNA is reactive and unstable bc of the 2'OH which makes it act like a nucleophile
        ii. DNA has nitrogenous bases A/T + G/C and RNA with A/U and G/C
    b. Function: DNA encodes all genetic info
    c. Common state: RNA has 3 types (mRNA, tRNA, rRNA)
    d. Synthesis: DNA polymerase requires 3' end of primer/DNA, dNTP
2. **Identify the bond broken when an NTP is added to a growing nucleic acid.**
    a. The bond between the alpha and beta phosphates
3. **Identify a glycosidic bond.**
    a. Between base pair and ribose
4. **Given the structure of a nucleotide, identify the type of nucleotide (purine or pyrimidine).**
    a. Pure As Gold - purine, AG
    b. A dumb way to remember that purines are two ringed is that you can get married if you are pure as gold, and in marriage, there are two rings
    c. Pyrimidines are one ringed, purines are two ringed
5. **Diagram and explain the structural and functional differences between the major and minor grooves of a DNA double helix.**
    a. Both
        i. Represent which side of glycosidic bond you are looking at
        ii. Every base pair has a major and minor groove side
    b. Major groove is longer path between R groups, minor groove is the shorter path
        i. hydrogen bond acceptors are all over the place, so good for specific binding
    c. Minor groove hydrogen bond acceptors are in the same place, so good for non-specific binding
        i. Shorter distance
6. **Identify the differences between the +1 base of mRNA and the START codon.**
    a. +1 base is the initiation site for transcription, while the START codon is the initiation site for translation
7. **Identify the sequential order of the promoter, +1, transcription terminator, ShineDalgarno sequence (aka RBS), start codon, and stop codon of a Protein-encoding gene in bacteria. Identify and order the elements required for a gene that encodes a functional RNA.**
    a. Promoter, then +1, then RBS, then start codon, then stop codon, then transcription terminator
8. **List the types of eukaryotic mRNA processing and identify the timing and cellular location of those processing events.**
    a. 5' G cap - methylated G protects from phosphatases
    b. Intron splicing
    c. Polyadenylation - AAUAAA
9. **Describe how bacterial transcription is regulated by repressors and activators.**

Commented [1]: dumb way to remember this, two people who are pure as gold get married, so two rings

    a. Upstream repressor gene (e.g. lac I) encodes repressor, which represses a downstream promoter. When the inducer (lactose) is present, it binds the repressor so that it cannot bind to the downstream promoter anymore.
    b. Activators binds to promoter and increases affinity of RNAP to promoter, e.g. only binds when glucose is scarse

10. **Predict the likelihood of RNAP binding and/or function in the presence of repressors and activators and in the context of mutations in binding sites for repressors and activators.**
11. **Describe how bacterial transcription can be regulated by a riboswitch.**
    a. Riboswitch - similar to hairpin, terminates transcription. Only occurs on environmental factors (metabolites)
12. **For an RNA with a riboswitch, diagram the structural feature(s) of a nascent RNA that contribute to transcription termination.**
    a. Stem loop, e.g. when enough riboflavin is made, then stem loop, else, no stem loop
13. Predict the relative amount of transcription that would occur based on whether a promoter region is found in euchromatin or heterochromatin.
14. **Describe the role of enhancers in transcription.**
    a. Enhancers increase transcription in eukaryotes
15. **Compare and contrast bacterial and eukaryotic transcription including a. initiation requirements b. polymerases used c. elongation and processing d. termination mechanisms**
16. **Describe the basics of Sanger (dideoxy) DNA sequencing**
    a. Sanger method used to sequence DNA
    b. Use DNA polymerase with dNTP and ddNTP, where ddNTP are double deoxy are fluorescent and therefore stop further replication and can be detected using a laser
    c. Run on a column, and smaller ones will elute out quicker, and you can detect the ones that come out.
17. **Interpret a Northern blot to assess size and quantity of RNA as well as gene regulation mechanisms and/or RNA stability.**
    a. Heavier, the less the RNA moves
18. **Compare and contrast Northern Blotting and Western Blotting.**
    a. Northern Blotting for RNA, Western Blotting for proteins, both are based on size
19. **Evaluate nucleic acid size and abundance based on the results of agarose gel electrophoresis.**
    a. Smaller molecules move faster
    b. How dark/wide a band is can tell you abundance
20. **Identify and describe the purpose of all of the components required for a PCR reaction including template, primer, thermostable polymerase, dNTPs, and Mg2+.**
    a. Goal of PCR is to copy a specific segment of DNA
    b. Template DNA
    c. Primer - DNA that is reverse complementary to sequence specific region, the region to make a copy of
    d. Thermostable polymerase - remains active at temperatures near the boiling point
    e. dNTP - serve as substrate
    f. Mg2+ - required for polymerase activity, stabilizes negative charge of phosphates in active site

g. Denaturation - separate strands by heating
h. Annealing - let primer bind by slowing cooling
i. Extension - increase temperature to let polymerase run
j. By two runs, you have the DNA region you want.

21. **Explain why the accumulation of product in a PCR reaction is exponential.**
    a. Synthesis of one strand can be used in template as the other, which is why it is called chain reaction. Copies can be copied.
22. **Apply knowledge of molecular biology techniques to a given experimental situation.**
    a. ChiP - used to find out where RNAP binds to. Cross-link proteins together to get all the protein and DNA together, then cut it all apart. Separate RNAP segments, then separate RNAP from DNA
23. **Explain the function of each of the following in DNA Replication a. helicase b. DnaA c. ssBP d. DNA Polymerases III and I e. proofreading exonuclease f. Ligase**
    a. Helicase - separates the strands
    b. DnaA
    c. ssBP - single stranded binding protein stabilizes the loop
    d. DNA polymerase III - adds dNTPs (both lagging and leading strands)
    e. DNA polymerase I - adds dNTPs between okazaki fragments (lagging strand)
    f. Proofreading exonuclease - removes mistakes, then replaces that region
    g. Ligase - seals gaps by forming bonds between fragments of lagging strand
24. **Predict likely consequences if any important molecules/steps in DNA replication were absent or very slow.**
25. Compare and contrast eukaryotic and bacterial translation initiation.
    a. In bacterial, binds at ribosome binding site and start codon
    b. In eukaryotes, binds to 5' end
26. **Explain the roles of rRNAs, tRNAs, and ribosomal proteins in translation.**
    a. rRNAs are a part of ribosomes
    b. tRNAs are used to translate codon/anticodon to amino acid after aaRS
    c. Ribosomal proteins contain three sites and
27. **Identify the type of enzyme/complex/molecule that "knows" the genetic code.**
    a. tRNA anticodon? mRNA?
28. Explain how the structure of the aaRS editing site contributes to the addition of the correct amino acid to a tRNA.
    a. There are some measures of accuracy in translation. Amino acids need to fit into the binding site. If they fit into the binding site, there is a chance they might still not be correct, so the editing site double checks that it is correct.
29. **Given a wildtype and mutant protein sequence, identify the type and location of mutations in the corresponding gene.**
    a. Indel slippage because of repeated sequences
    b. Mismatches because of tautomerization
    c. Lesions because of mutagens
30. **Design a mutation in a DNA sequence that will encode a mutant protein.**
31. **Explain the potential consequences of an error in DNA replication, DNA repair, transcription, and translation on an encoded protein.**

32. Predict the effect of mutations that would affect the function of encoded RNAs or proteins.