MCD Level 1 Exam 2014

Part 1 General Essay: This section of the exam is worth 60% of the entire grade. (Answer any **four** of the next six questions- do not answer all six- only answer four. Make sure to develop your thoughts with fully developed sentences. This is a general essay that must answer the questions put forth below. Each question is worth 15 points.)

1. (*1, 2*)

Lipid droplets are organelles for the storage of cellular fats. Recent evidence has demonstrated that lipid droplets are dynamically regulated to coordinate the storage and release of lipids in response to cellular and environmental conditions.

A. Describe the structure and composition of lipid droplets. What types of lipids and proteins are present in these organelles, and how are they localized within the lipid droplet? (5 points)

B. In *C. elegans*, mutants defective in any of the three genes *maoc-1, dhs-28* or *daf-22* have alterations in lipid droplet structure. The protein products of these genes function in the same biochemical process; what is this process? (3 points)

C. What happens to lipid droplet structure in *maoc-1, dhs-28* and *daf-22* mutants? How were these defects identified? (5 points)

D. What are the advantages and disadvantages of studying lipid droplets in the nematode *C. elegans*? (2 points)

2. (*3, 4*)

HIV is a major heath concern worldwide and it has proven difficult to generate an effective vaccine to this pathogen because of its high rate of mutation. While highly active retroviral (HART) therapy has proven to be very effective in treating AIDs patients, many individuals do not tolerate this well and this medication has a short half-life. Recently, broadly reactive neutralizing antibodies (bNAbs) to HIV have been re-examined as a possible therapeutic strategy to combat AIDs.

A. Describe what neutralizing antibodies are and how they may be used as an effective vaccine for the treatment of HIV. What are the targets of various bNAbs to HIV? (5 points)

B. What is the challenge in generating neutralizing antibodies to HIV? Briefly describe how rational design of the HIV viral envelope can facilitate the identification of broadly reactive neutralizing antibodies. (5 points)

C. How can HIV escape being targeted by a single neutralizing monoclonal antibody? What advantage does a combination of monoclonal antibodies have over a single monoclonal antibody as a vaccine for HIV? (5 points)

3. (*5-7*)

The Cre/lox system has been widely used in mice for many applications including targeted gene expression and tissue-specific knockouts. It has recently been used in zebrafish to trace the lineage of specific cells during organ regeneration.

A. Explain how the Cre/*lox* system works. (4 points)

B. Describe how researchers used this system to trace the lineage of the cardiomyocytes involved in heart regeneration after amputation in zebrafish. Did they originate from stem cells? (4 points)

C. If regeneration is not observed in the mammalian heart, why is it useful to understand how regeneration occurs in zebrafish? (3 points)

D. Heart injuries in mammals are generally associated with cell death due to ischemia. How was it determined if zebrafish heart regeneration could overcome this obstacle? (4 points)

4. (*8, 9*)

Spemann received the Nobel prize for uncovering the role that tissue "organizers" play during early development. Organizers are found spatially abutting fields of undifferentiated, naïve precursor cells. Organizers initiate and direct the orderly diversification of these naïve cells. 70 years after Spemann's seminal observations in Xenopus the first vertebrate morphogens were discovered.

A. What is a morphogen (5 points)?

B. Taking early neural tube development as an example, describe how morphogens "pattern" naïve tissues (10 points)?

5. (*10-12*)

Programmed cell death (apoptosis) plays a critical role in multiple biological events including development, tissue homeostasis, and elimination of harmful cells, including tumor cells. The early removal of apoptotic cells is also essential to avoid the leakage of immunoreactive materials from dying cells. This process is preferentially carried out by professional phagocytes, such as macrophages and monocytes. A recent study by Konishi et al. indicates that a critical autophagic protein, Beclin 1, plays an important role in phagocytosis of apoptotic cells (Konishi et al.; 2012). Using Beclin 1-deficient embryonic stem cells as well as other cells in which Beclin 1 was knocked-out by shRNA, the authors showed that Beclin 1 is recruited to the early phagocytic cup along with Rac 1, which regulates actin dynamics and lamellipodia.

A. Briefly describe the two major signaling pathways leading to programmed cell death (PCD) or apoptosis in mammalian cells. (4 points)
B. Provide one specific example of organogenesis and one specific example of tissue remodeling involving apoptosis. (4 points)
C. Describe the molecular pathway of apoptosis and the molecular pathway of autophagy. (5 points)
D. What are the main differences? (2 points)

6. (*13, 14*)

A. Explain the primary function of the DNA mismatch repair system. Include an explanation of how this pathway maintains genome integrity and also discuss the basic DNA mismatch repair mechanism in a prokaryotic system. Define the substrates and proteins involved, including all types of DNA fragments involved in the reaction. You must provide sufficient mechanistic detail of the reaction to insure that your explanation is clearly communicated. (8 points)

B. Include in your essay an explanation of how the **prokaryotic** repair system identifies which strand contains the original sequence and which contains the mismatch. How do you think the **eukaryotic** repair system identifies which strand contains the original sequence and which stand contains the mismatch? If this information is not known, state so and speculate as to why it may not be known. (4 points)

C. Given that there are several other repair pathways active in most cells, why is this DNA repair pathway needed? Is there a hierarchy of activity for DNA repair pathways? Discuss why or why not. (3 points)

Part 2A Experimental Design: This section of the exam is worth 20% of the total grade. (Answer **either** question 7 **or** question 8-do not answer both.)

7. (*15-17*)

Phagocytosis plays a major role in the defense of higher organisms against microbial infection and provides the basis for antigen processing in the immune response. Cells of the model organism *Dictyostelium* are professional phagocytes that exploit phagocytosis of bacteria as the preferred way to ingest food, besides killing pathogens. *Dictyostelium discoideum* can be grown in the presence of their preferred food source, bacteria, or in a defined medium in the absence of bacteria. In the presence of bacteria, the cells use receptors to bind bacteria and engulf them using phagocytosis. In order to better understand bacterial recognition, you are interested in determining whether the *Dictyostelium* cells use different receptors on their cell surface depending on whether they are feeding on gram$^+$ or gram$^-$ bacteria.

A. How would you determine whether *Dictyostelium* cells grown on gram$^+$ bacteria express different cell surface proteins than *Dictyostelium* cells grown on gram$^-$ bacteria? (5 points)

B. You identify hundreds of proteins that are differentially expressed on the cell surface in the presence of the different bacteria. How would you narrow this list down to a few potential receptors? (5 points)

C. You narrow your list down to two uncharacterized proteins that are expressed on the cell surface only when *Dictyostelium* cells are grown on gram$^+$ cells. You name them PosA and PosB. Propose an experiment to determine whether PosA and/or PosB are required for *Dictyostelium* cells to feed on gram$^+$ cells. (5 points)

D. You determine that PosA is required for *Dictyostelium* cells to feed on gram$^+$ cells. PosA could be required to bind gram$^+$ bacteria. However, it is also possible that PosA has nothing to do with binding gram$^+$ bacteria, but is required to digest gram$^+$ bacteria. What experiment could you perform to determine which is the function of PosA? Draw and propose one outcome for the result of this experiment (either PosA is required to bind gram$^+$ bacteria, or PosA is required to digest gram$^+$ bacteria). The result may take the form of a graph. Include a control. (5 points)

8. (*18-20*)

You are studying a region of DNA (region X) that lies in between two genes (Gene Y and Gene Z). In whole animals, Gene Y's expression pattern is very different from that displayed by Gene Z, indicating that two distinct gene expression programs are operating on either side of region X. You strongly suspect that region X is playing a key role in keeping the influences of the Gene Y and Gene Z regulatory programs separated. This would prevent the Gene Y program from interfering with the Gene Z expression program, or vice versa. In cell type W, Gene Y is highly expressed while Gene Z is silent. Please answer the following:

A. Describe two possibilities for the type of regulatory activity being supported by region X. Briefly discuss the role of at least one key protein involved in each of the regulatory activities you describe. (5 points)

B. Design the reporter constructs required to test a cloned piece of region X DNA for the two possible activities described in A. Please include the appropriate control constructs you will use in your tests of each of your reporter constructs in cell type W, which is easily transfected. (10 points)

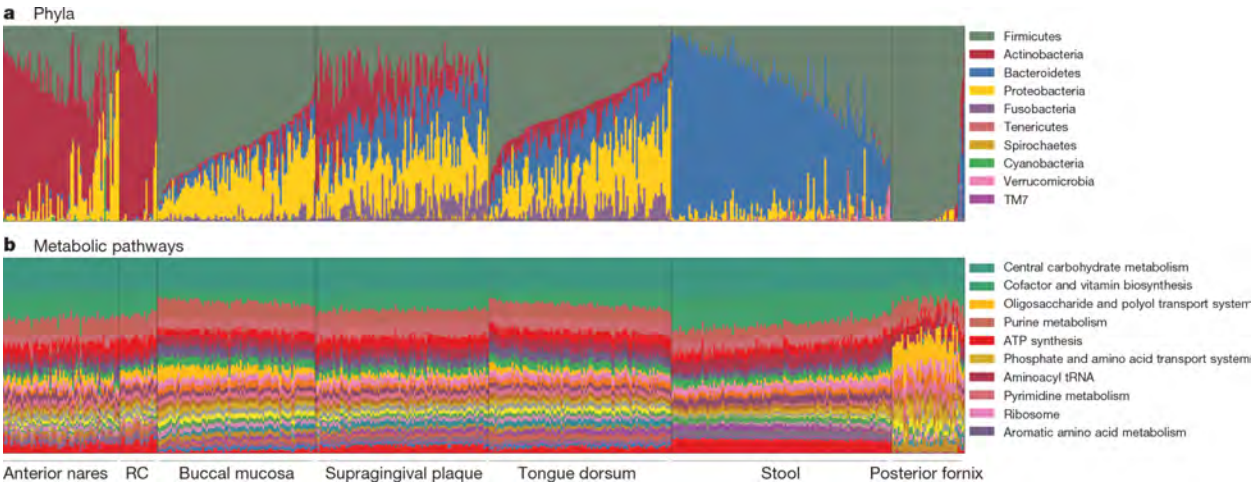
C. Please discuss the range of possible results for the above experiments you designed. Please relate these results to the putative regulatory role of region X in the native Gene X/Y locus. (5 points)

Part 2B Data Interpretation: This section of the exam is worth 20% of the entire grade. (Answer **either** question 9 **or** question 10- do not answer both.)

9. (*21, 22*)

This question concerning the human microbiome is based on data presented in a recent article: Structure, function and diversity of the healthy human microbiome (*Nature*, June 2012, from The Human Microbiome Project Consortium).

A. The difference in the relative abundance of various phyla across individuals is significant (Figure 1a). What factors may contribute to this diversity in humans? What implications does this diversity have regarding human health and disease? Use specific examples from below in your response. (7 points)
B. Describe the data shown in panel b (below) and propose a testable hypothesis based on the data shown in panels a and b (3 points).
C. Based on the data presented in the figure below, what conclusions can you draw regarding the significance of the diversity of bacterial phyla across individual human microbiomes and the metabolic diversity across individual human microbiomes? (10 points)



10. (*23, 24*)

The main bacterial pathway of DNA double-strand break (DSB) repair involves a mechanism in which one or both of the DSB ends is resected by a nuclease to leave a short single-stranded DNA tail. This resection of DSBs is a motor-driven process performed by a multisubunit helicase–nuclease complex. The results shown in the figure below are from a group of researchers characterizing the single-strand DNase (ssDNase) activity of a helicase–nuclease complex (ApcUB) involved in resection of DSBs in a newly discovered deep-sea bacterium. Please analyze the results shown and answer the following questions.

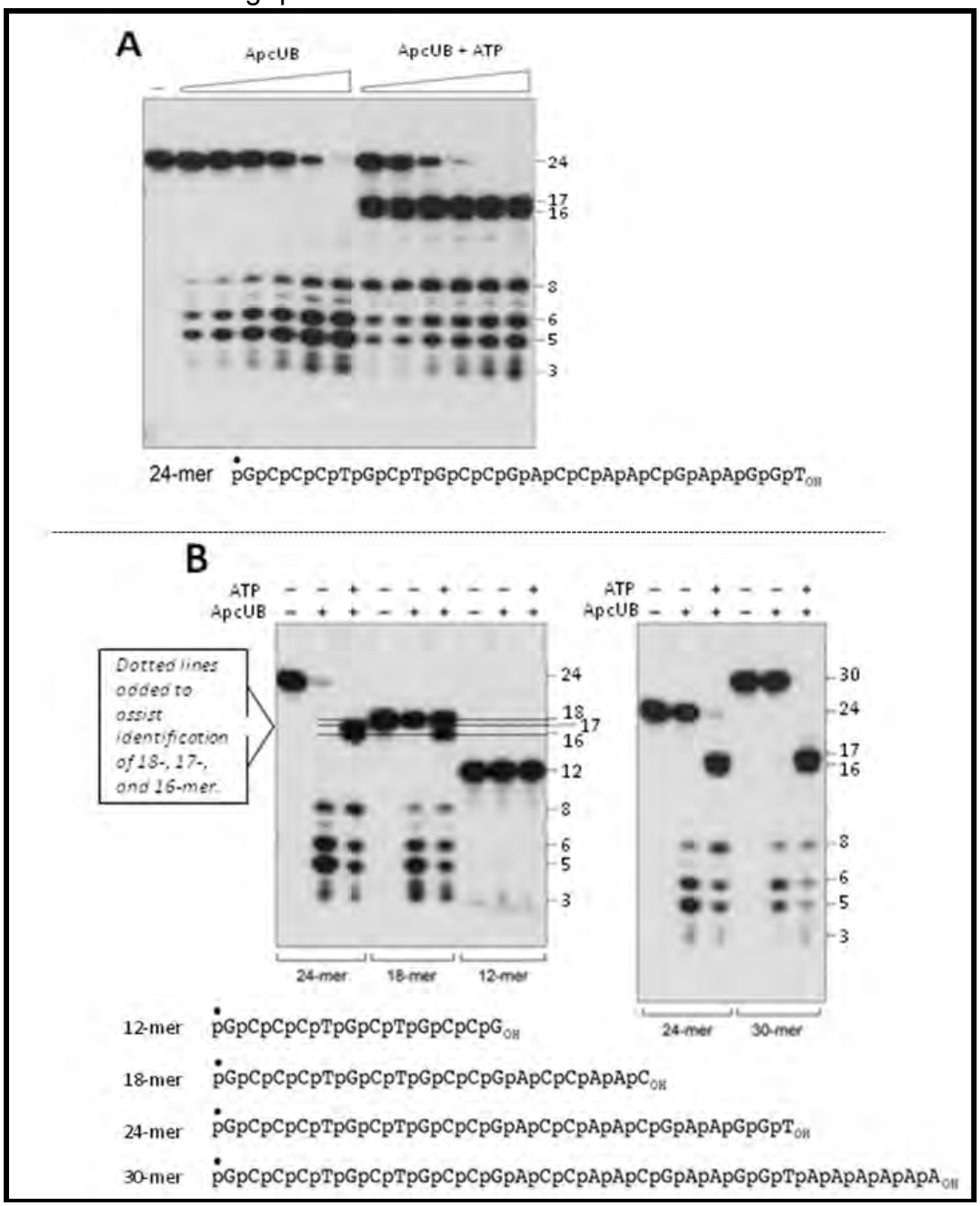


Figure Legend. ssDNase activity assay for ApcUB with 5'^{32}P-labeled single strand DNA substrates. (A) Reaction mixtures containing 0.1 μM 5'^{32}P-labeled 24-mer oligonucleotide (shown in the figure), either no ATP or 1 mM ATP, and purified ApcUB (in the amount of 1, 2, 3, 5, 10, and 20 ng from left to right in each titration series) were incubated for 10 min at 20°C. ApcUB was omitted from the control reaction (left lane). The reaction products were analyzed by urea-PAGE and visualized by autoradiography. (B) Reaction mixtures containing 0.1 μM 5'^{32}P-labeled 30-mer, 24-mer, 18-mer, or 12-mer oligonucleotide substrates (depicted in the figure); 1 mM ATP (where indicated by +); and ApcUB (10 ng, where indicated by +) were incubated for 10 min at 20°C. Reaction products were analyzed by urea-PAGE and autoradiography. The numbers on the right of the autoradiography images indicate the length in nucleotides and positions of 5'^{32}P-labeled oligonucleotide substrates and main reaction products. The dot above the 5' phosphate of each displayed oligonucleotide substrate represents the ^{32}P radiolabel. Please note that the overlapping segments of the oligonucleotide substrates have exactly the same sequence.

A. Based on the results shown in Panel A, what are the two effects that the presence of ATP has on the nuclease activity of ApcUB? For your analysis, please consider only the main products marked on the right of the autoradiography image. *(6 points out of 20)*

B. Based on the patterns of cleavage shown in Panel B, what can be concluded about the nuclease activity of ApcUB? For your analysis, please consider only the main products marked on the right of the autoradiography images. *(14 points out of 20)*

2014 MCD Reading List:

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14. Acharya, S., Foster, P. L., Brooks, P., and Fishel, R. (2003) The coordinated functions of the E. coli MutS and MutL proteins in mismatch repair, *Molecular cell 12*, 233-246.
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