

MCD Level 1 Exam 2013

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. The references for each question are shown after each question number and can be found at the end of the exam.

1. (1, 2)

- A. What are “induced pluripotent stem cells” (IPS cells) and how do they differ from embryonic stem (ES) cells ? 4pt
- B. What is nuclear reprogramming? Prior to the derivation of IPS cells, how was nuclear reprogramming achieved ? 5pt
- C. What is the therapeutic purpose of nuclear reprogramming or IPS cells? 3pt
- D. Discuss one major therapeutic limitation of IPS technology. 3pt

2. (3-6)

Type II transcription units (non-tRNA, non-rRNA genes) are the basis for most genes in eukaryotic organisms.

A. Starting with a gene, briefly explain the key steps by which an mRNA is produced, exported and ultimately translated into a functional protein. 5pt

B. Briefly describe the essential elements of a gene as currently accepted and contrast the early definition of a gene with the current definition based on the ENCODE article. 5pt

C. Please describe three different kinds of Type II transcription units in eukaryotes that would classify as distinct genes. As part of your description, outline the variety of mRNAs that are produced. 5pt

3. (7)

In nature, the lack of nutrients is a common threat for all living organisms. Some organisms such as bacteria and plants can come together and help each other to survive the lack of nutrients. Drawing from Jones et al. (“How rhizobial symbionts invade plants: the Sinorhizobium–Medicago model”):

- A. Describe how each partner benefits from this interaction. 5pt
- B. At which step of these bacterial-plant interactions do the partners need to recognize each other? 5pt
- C. In a few sentences, please describe the physical interactions. What is the molecular basis that allows the partners to selectively recognize each other? 5pt

4. (8-10)

The asymmetric localization of messenger RNA (mRNA) and protein determinants plays an important role in the establishment of complex body plans. In *Drosophila* oocytes, the anterior localization of *bicoid* mRNA and the posterior localization of *oskar* mRNA are key events in establishing the anterior-posterior axis.

- A. Name and define 3 mechanisms of RNA localization. (4 points)
- B. Describe one method for detecting and tracking mRNAs in living cells. Identify limitations for this technique. Explain clearly using a diagram if necessary. (4 points)
- C. What is the experimental evidence that splicing plays a role in the regulation of *oskar* mRNA localization? (3 points)
- D. Using a diagram, identify the main players involved in the transport of *oskar* mRNA from its site of transcription in the nucleus to the posterior pole of the oocyte. (4 points)

5. (11-13)

Myelin sheath is specifically made by Schwann cells and by oligodendrocytes in the peripheral (PNS) and central (CNS) nervous systems respectively.

- A. Briefly discuss the adaptive advantage that myelin confers to vertebrate nervous systems. (3pt)
- B. What are the main consequences of demyelination of axons? (3pt)
- C. Describe the key experimental findings (*in vitro* and *in vivo*) made by Lee et al., "Oligodendroglia metabolically support axons and contribute to neurodegeneration", supporting a direct role for oligodendrocyte MTC1 in the pathogenesis of amyotrophic lateral sclerosis (ALS). In your answer please refer to: pattern of MTC1 expression, functional consequences of MTC1 downregulation/ablation and the effects of glucose deprivation/lactate supplementation. (6pt)
- D. According to the author's model (Lee et. al.) : how can physical activity benefit patients with neurodegenerative diseases? (3pt)

6. (14, 15)

Based on your readings of Feng et al. (Live Imaging of Tumor Initiation in Zebrafish Larvae Reveals a Trophic Role for Leukocyte-Derived PGE₂) and Mione and Zon (Cancer and Inflammation: An Aspirin a Day Keeps the Cancer at Bay):

- A. What are 3 differences between innate and adaptive immunity? (3 points)
- B. What is inflammation and how is it linked to cancer? (2+2 points)
- C. What makes zebrafish an attractive and unique model system to study linkages between cancer and inflammation? (4 points)
- D. What is the principle behind the use of Morpholinos and why were Morpholinos used in Feng et al., 2012 study? (2 points)
- E. What are eicosanoids? What is the subcellular location of the PGE₂ receptor in zebrafish cells? (2 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. (16, 17)

In the Lee et al. paper, the authors proposed a novel mechanism by which *lin-4* regulates *lin-14* expression in *C. elegans*; this mechanism has now been found in many other organisms.

- A.** (3 pts) Describe the *lin-4* gene. What does it encode? How is this product processed? What is (are) the phenotype(s) of *lin-4* mutants?
- B.** (3 pts) If there is a point mutation in *lin-4*, describe how this mutation could affect *lin-4* activity. What sort of point mutation would NOT affect *lin-4* activity?
- C.** (6 pts) Given the current knowledge about the mechanism of *lin-4* (described in the review article by Ambros), design an experiment to show that *lin-4* regulates *lin-14*. Describe the pathway and draw a diagram to illustrate the mechanism.
- D.** (8 pts) Suppose that you hypothesize that there are other regulatory targets of *lin-4*. What sort of targets would you predict? What mutant phenotypes of these target genes would you expect? What experimental criteria would you use to find these target genes? Describe experiments that would demonstrate that these putative targets are actually *lin-4* targets.

8. (18, 19)

- A.** Bacteria are not simple “bags” of enzymes but have a rich and diverse cytoskeleton. Discuss the above statement in the context of your readings. (6pts)
- B.** The metabolic enzyme CTP synthase forms filaments in bacteria, yeast, and in fruit flies indicating that polymerization is a conserved feature of the enzyme. In bacterial species such as *Caulobacter* this enzyme is bifunctional, as the filaments regulate cell shape independent of its catalytic activity. However, in bacteria such as *E. coli*, the function of the polymerization activity of the enzyme remains unclear. Design a set of well-controlled experiments that would allow you to test the hypothesis that polymerization regulates enzymatic activity of CTP synthase. (14 pts)

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. (20)

You mutagenized yeast haploid cells, and isolated three yeast haploid mutants which are defective in cell division. These mutants were named *cdm* for cell division mutant (*cdm1*, *cdm2*, *cdm3*). When these cells are incubated at the non-permissive temperature (37°C), they cannot divide and show lethality indicating that these are temperature sensitive mutants. You have decided to follow up on these mutants with few simple classic genetics tests. Here are your results.

A. You crossed the *cdm1* or *cdm2* mutant with a wild type haploid strain. The resulting diploid shows wild type phenotype in both cases. What can you conclude from this result about the *cdm1* mutation and the *cdm2* mutation? What type of mutation do they have? (4pts)

B. You crossed one of these mutants (*cdm3*) with a wild type haploid strain. The resulting diploid showed the mutant phenotype. The diploid cells could not grow at the non-permissive temperature. What can you conclude from this result about the *cdm3* mutation? (4pts)

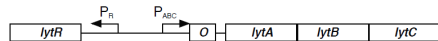
C. You cross two haploid mutants with each other, *cdm1* and *cdm2*. The diploid showed the mutant phenotype at 37°C. What do you conclude from this result? (4pts)

D. A previously identified *tub1* haploid mutant (*tub1-1* is a recessive mutation on the tubulin gene) was crossed with *cdm1* mutants. Tetrad dissection was performed using spores after mating. You noticed that PD:NPD:T ratio was 9:0:0. What do you conclude from this result? (4pts)

E. You incubated the *cdm1* mutant at 37°C and observed it under the microscope. Please draw the phenotype of the cells, highlighting the chromosomes and spindles. (4pts)

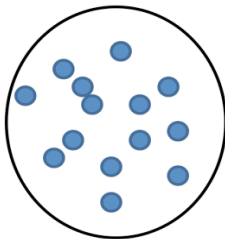
10. (21) An outbreak of food poisoning causing acute kidney failure was traced to a new pathogenic strain of *E. coli*. Via whole genome sequencing, the *E. coli* strain was found to be almost identical to a commensal *E. coli* strain except for the insertion of a novel phage, K2, in its genome. The CDC has sent the phage to you and asked for your help in characterizing this new health threat.

A critical region of the phage genome is diagramed below.

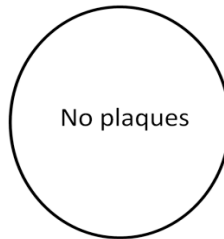


Preliminary studies indicate that the *lytR* gene encodes a protein that regulates the expression of an operon containing the *lytA*, *lytB*, and *lytC* genes. *lytA* and *lytB* are required for the lytic life cycle of the bacteriophage since phage lacking these genes are unable to productively infect *E. coli*. The plaques formed on *E. coli* by normal K2 phage and the lack of plaques in *lytA* and *lytB* mutants are shown below.

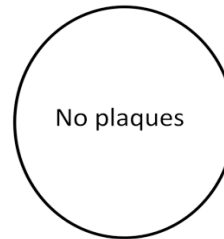
E. coli infected with K2 at 42°C



E. coli infected with K2/*lytA*- at 42°C

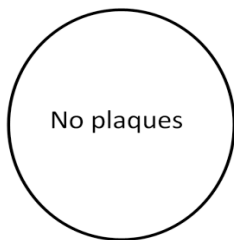


E. coli infected with K2/*lytB*- at 42°C

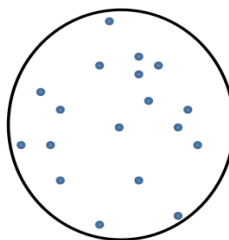


It is your job to characterize the regulatory region of this critical region of the phage genome. Recent reports show that kidney failure is more likely to occur in patients with severe fevers than in patients without fevers. Based upon these reports, you decide to infect *E. coli* with the phage at different temperatures to determine if there are any differences in phage growth. The plaques formed in each case are shown below (in blue).

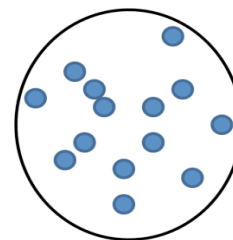
E. coli infected with K2 at 30°C



E. coli infected with K2 at 37°C



E. coli infected with K2 at 42°C

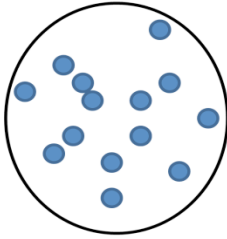


1) If the *LytR* protein negatively regulates the *lytABC* operon, what would you expect to happen to *lytABC* expression when *lytR* was deleted? Why? (4pts)

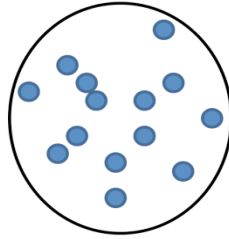
2) If the *LytR* protein positively regulates the *lytABC* operon, what would you expect to happen to *lytABC* expression when *lytR* was deleted? Why? (4pts)

You construct a deletion mutant that is lacking the entire *lytR* gene. You then repeat your temperature experiment and obtain the following results:

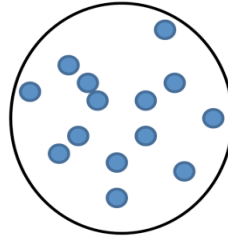
E. coli infected with K2del(LytR) at 30°C



E. coli infected with K2del(LytR) at 37°C



E. coli infected with K2del(LytR) at 42°C

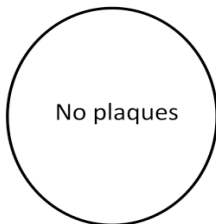


3) From these results, do you suspect that LytR regulates the operon positively or negatively? Why? (4pt)

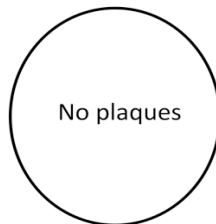
4) Other researchers have shown that the *lytR* gene is expressed constitutively. Based upon what you have learned about LytR so far, suggest a model for how the LytR protein functions to regulate *lytABC* expression at 30C? at 42C? (4pts)

A colleague tells you that almost all of the *lytR* mutations that she has isolated behave exactly like a *lytR* deletion in the assay shown above. However, she has one mutant which she calls *lytRind* that gives her the following results in this assay.

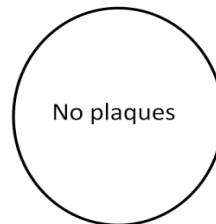
E. coli infected with K2LytR^{ind} at 42°C



E. coli infected with K2LytR^{ind} at 42°C



E. coli infected with K2LytR^{ind} at 42°C



5) What kind of mutation is *lytRind* likely to be and how does that result in the observed phenotype? (4pts)

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