MCD Level 1 Exam 2015

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***

Most of the driver mutations in human cancer are in genes that encode proteins that regulate G1 cell cycle progression. There has been much confusion on the key checkpoints that govern progression from mitosis through G1 and into S-phase, where the cell commits to replicating the genome and dividing. The most controversial point is the restriction point (R): Some have put R in early G1 where cyclin D is active and others have put R late in G1 where cyclin E is active.

a) Explain the times in the cell cycle when cyclins D and E are active, what is monitored by the cyclin D- and cyclin E-mediated checkpoints, and what are the major effectors. (5 points)
b) Explain how Ras-driven cancer cells helped to establish the existence of a late G1 checkpoint that is responsive to the presence of glutamine. (5 points)
c) How does interfering with anaplerotic utilization of glutamine provide therapeutic opportunities against Ras-driven cancer cells. (5 points)

2. ***3, 4***

Epigenetic modifications of the DNA and histones (as well as other mechanisms) occur in somatic cells. Loss of most of these modifications needs to occur to establish a new generation.

a. Discuss the cycles of germline reprogramming in mice, focusing on DNA methylation. Indicate what cells are involved in each process and what types of DNA escape. Indicate how this relates to the possibility of human epigenetic inheritance. (4 points)

b. The review from Heard and Martienssen described transgenerational epigenetic inheritance. Pick 2 of the following mechanisms for transgenerational epigenetic inheritance and articulate a detailed example of a proposed mechanism and one or more systems that have been studied. (7 points)

a. RNA interference
b. RNA-dependent DNA methylation
c. Paramutation
d. Histone modification

c. Describe the main experimental results in the paper by Kerr et. al.(4) about the cooperation of the *spr-5* and *met-2* genes in *C. elegans*. (4 points)

3. ***5, 6***

The levels of intracellular proteins are regulated by the rate of their synthesis as well as by the rate of their degradation. The two major pathways for intracellular protein degradation are the ubiquitin/proteasome pathway and the autophagy/lysosomal pathway. Based on your readings of Lilienbaum (Relationship between the proteasomal system and autophagy) and Lee et al. (Tau degradation: The ubiquitin-proteasome system versus the autophagy-lysosome system):

(A) Compare both pathways in terms of the degradation machinery. (5 points)

(B) Compare both pathways in terms of substrate specificity and delivery. (5 points)

(C) Discuss therapeutic potentials for promoting the degradation of the cytoskeletal protein “tau”, which plays a critical role in Alzheimer disease. (5 points).

4. ***7, 8 9***

Bacteria rely on a number of strategies to escape or prevent infection and regulate defense. A new method for genome manipulation uses a system identified from a bacterial adaptive immune response, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR).

(A) How have bacteria adapted DNA repair mechanisms from damage to defense? (5 points)
(B) Describe how specificity is achieved in the CRISPR system and how this system is being used to direct genome editing in other organisms? (5 points)
(C) What are the implications of introducing the CRISPR components into a mammalian system? (5 points)

5. ***10, 11***

Membrane proteins need an effective mechanism to be targeted to the plasma or organelle membrane upon appropriate stimulation by external stimuli prior to being effective. Dysfunction results when membrane targeting/association is disrupted.
(A) Using the example of membrane-targeted APPL proteins, describe the specific molecular mechanism for targeting APPL1 and APPL2 to the membrane. (5 points)
(B) In the context of endosome-mediated signaling, what are the downstream biological processes mediated by their membrane based functions and what other proteins are involved? (5 points)
(C) How is membrane targeting of APPL1 modulated in insulin signaling cell types such as hepatocytes? Explain the role of this modulation on the mechanism underlying the insulin-sensitizing actions of APPL1 highlighting the key protein players involved. (2+3 points).

6. ***12***

(A) Mitochondria generate energy primarily through an initial step of pyruvate entering the mitochondrial matrix and ending with the generation of ATPs within the mitochondrial intermembrane space. Name the pathway that generates pyruvate. Name the two pathways that drive ATP production and describe the factor and process that interconnects both. Name the enzyme that generates the “bulk” of ATP. A number of other important pathways are found in the mitochondria, which do not lead to an accumulation of ATP – name three of these. (10 points).
(B) Mitochondria sense cellular stress and control intrinsic programmed cell death. The initial steps of this process involve breaching of the mitochondrial membrane, while the final steps involve pro-caspase activation. Describe the factors and processes that occur between the initial and final steps mentioned above (5 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. ***13, 14***

Familial “Green Martian Disease” (FGMD) is a heritable co-dominant trait in which affected individuals have a greenish tint to their skin. The trait has been mapped to the long arm of chromosome 18 where the gene for ferrochelatase resides. Dr. Harlee Nositall has hypothesized that in FGMD, a missense mutation in the enzyme ferrochelatase causes it to insert Mg++ instead of Fe++ into protoporphyrin IX. Dr. Nositall also hypothesizes that the Mg++-protoporphyrin IX is green, and is subsequently bound to globin chains in hemoglobin. The sequence of ferrochelatase is known, and you can buy human ferrochelatase cDNA from Invitrogen.

Using the two areas below state how would you test Dr. Nositall’s hypothesis?

A. How would you determine whether affected individuals have a mutation in the gene for ferrochelatase? (10 points)
B. Assuming you find such a mutation, how would you determine whether this mutation leads to synthesis of a green form of Mg++ hemoglobin? (10 points)

8. ***15, 16***

Background: The eight orthomyxovirus Influenza virus genome segments are packaged into a ribonucleoprotein (RNP) complex that includes four proteins, PB1, PB2, PA, and NP. Each of the proteins PB 1 – 3 is a subunit of an RNA-directed RNA polymerase that is specific for the replication of the eight negative strands of viral RNA. NP is a single-stranded RNA – binding protein that interacts with the polymerase subunits as well as RNA. Assembly and function of these proteins to form the entire RNP depends on a complex set of protein-protein and protein-RNA interactions. One particular interaction between the PB2 and NP polypeptides is thought to be responsible for the switching from viral mRNA to complementary RNA (cRNA) synthesis.

Data: In order to better delineate this interaction you will need to know how to design experiments that show which regions of PB2 actually bind to NP. Wild type PB2 protein is 759 amino acids and is encoded by a negative stranded viral RNA of about 2300 bases. Wild type NP protein is 498 amino acids in length encoded by a negative stranded viral RNA of 498 bases. Assume that there is no three dimensional (3-D) information derived by either crystallography or nuclear magnetic resonance studies. The overall experimental goal is to draw a linear map describing which regions of PB2 interact with NP in the hope that this information can simplify a future 3-D structural study.

Materials: The major reagents available are cDNA copies of both viral PB2 and NP RNA segments that have been cloned by insertion into plasmid pPrm, a typical *E. coli* and eukaryotic expression vector. You also have a cloning vector that carries the glutathione – sepharose binding protein sequence (GST). The labeled amino acid ^{35}S-methionine is available as well.

a. (5 points) Which recombinant protein should be labeled and why?

   Which recombinant protein should carry the GST amino acid sequence and where?

b. (5 points) How would the interaction of the PB2 and NP be detected?

c. (5 points) What mutations should be made in the target protein in order to locate the NP binding domains? (Summarize the mutants using a linear map.)

d. (5 points) Suggest the functional significance of the PB2 and NP interaction that you have demonstrated experimentally.

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. ***17, 18***

Based on your reading of Zhu *et al.* ("Cisplatin causes cell death via TAB1 regulation of p53/MDM2/MDMX circuitry"):

A. After having demonstrated the association between TAB1 and MDM2 by two-hybrid screening, why do the authors still need to verify the association between TAB1 and MDM2 in U2OS cells? (4 points)
B. Why do the authors conclude that TAB1 employs one or more additional mechanisms to contribute to cisplatin-induced cell death? Which experiments help them conclude this? (4 + 4 points)
C. Why is this work relevant even though the authors showed that the TAB1/p53/MDM2/MDMX pathway only applies for cisplatin treatment and not with a variety of different genotoxic assaults? (4 points)
D. In the hypothetical case of personalized therapy against cancer, and based on the evidence presented in this paper, what genes should be screened before treating patients using cisplatin? (4 points)

Figure 1 from the manuscript:

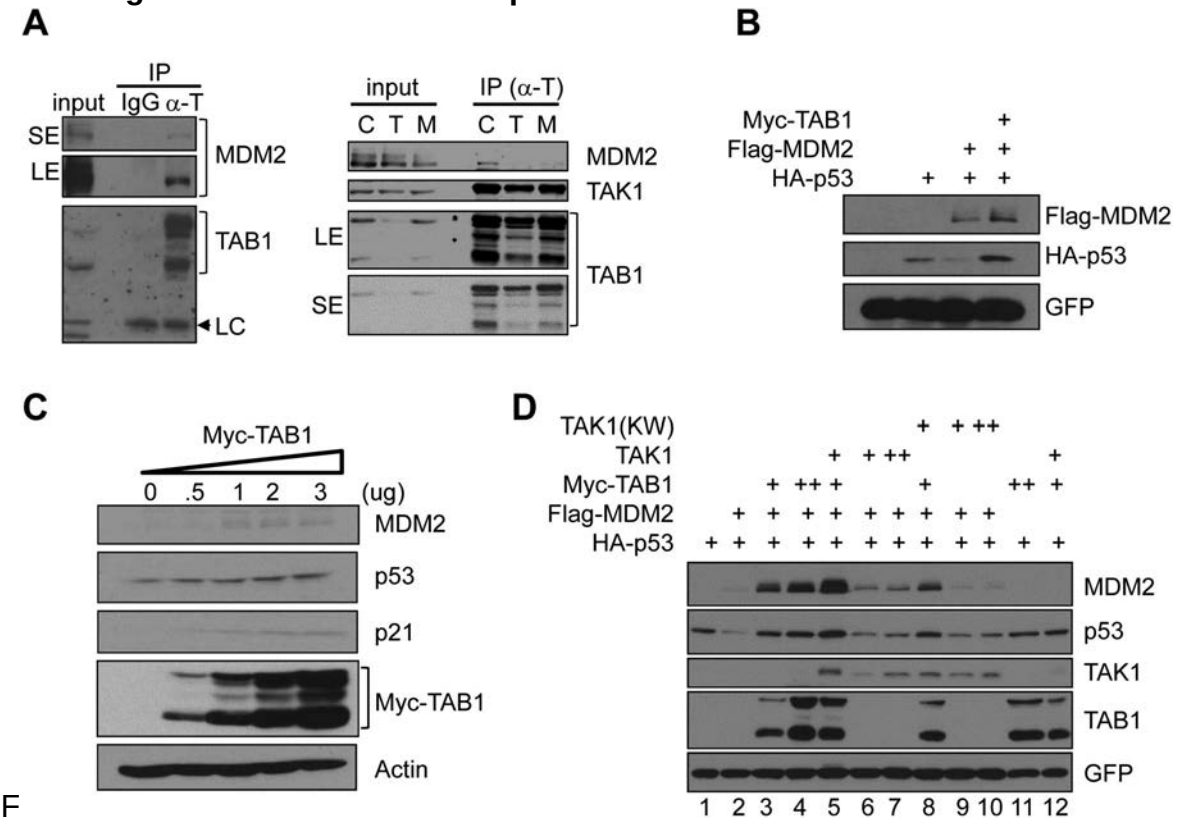

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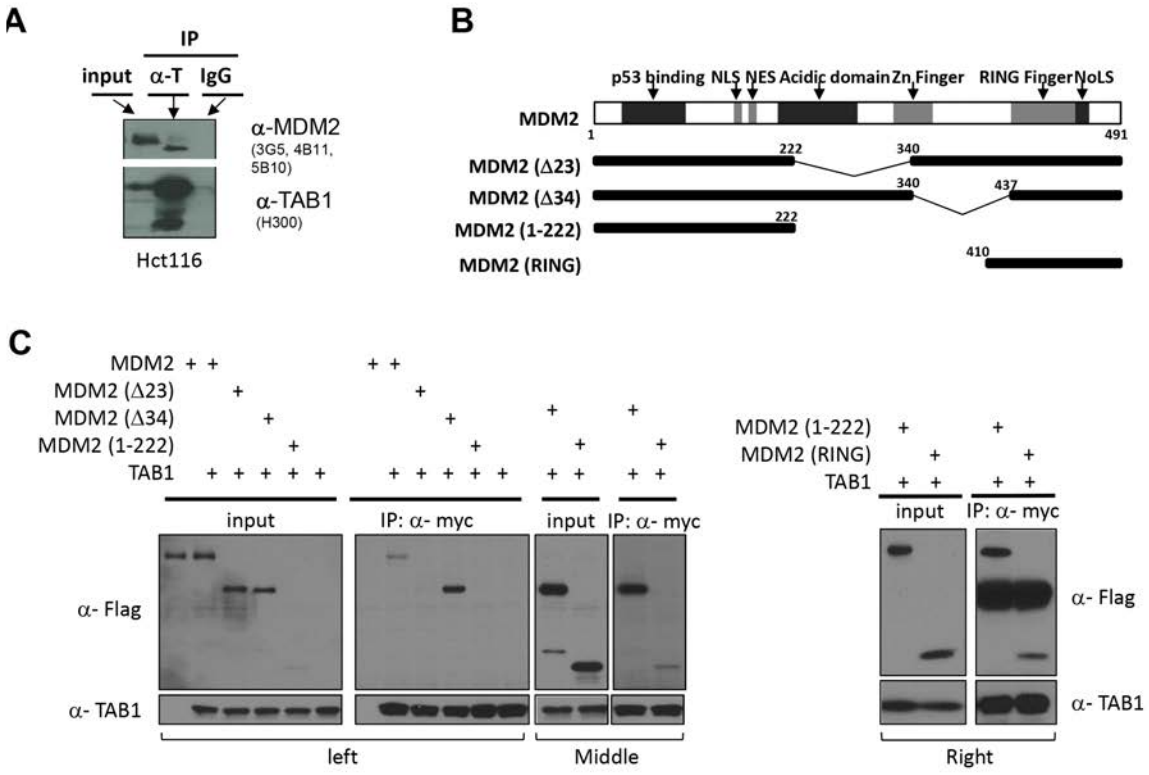
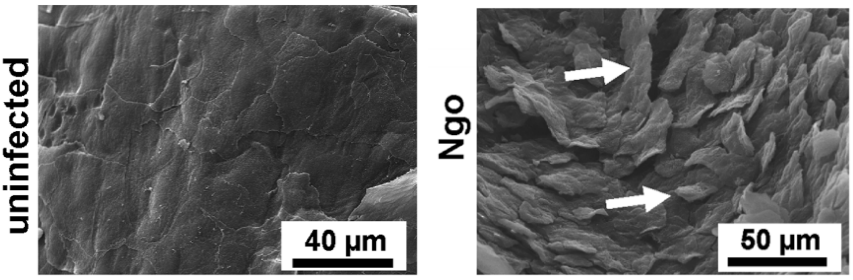
Figure S1 from the manuscript:



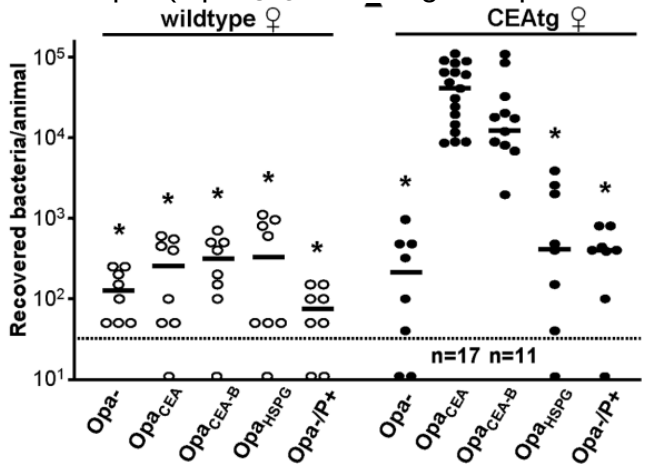
10. ***19 20***

In order to study the interaction of the bacterium *Neisseria gonorrhoeae* (Ngo) with human cells, researchers have developed a transgenic mouse model. These mice bear the human gene CEA (CEA transgenic mice or CEAtg).

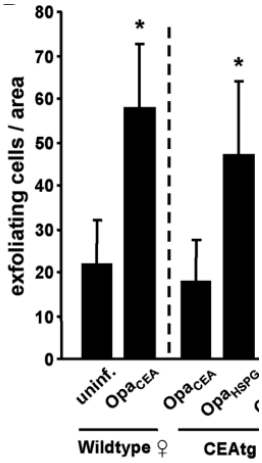
As a first experiment, scientists have infected Wild Type (WT) mice (not the transgenic ones) with Ngo cells bearing different types of a cell surface protein called Opa (either no Opa (Opa-) or Opa known to interact with different molecules. (For instance, Opa_{CEA} is know to interact with the human CEA receptor). Below is a picture of genital epithelia from female wt mice either uninfected or infected with Ngo. Describe and interpret these images and discuss the impact of Ngo infection on WT mice genital epithelia. (4 points)



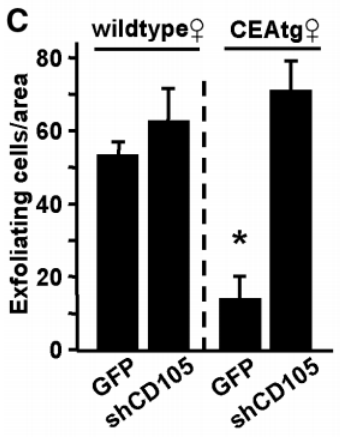
A. The graph below characterizes the number of living bacteria that could be recovered from the urogenital region of mice infected with 10^8 bacteria the previous day. Summarize briefly the results of the graph and draw a conclusion of the interaction between the human CEA gene and the bacterial membrane protein Opa. (Opa_{HSPG} is binding to heparan sulfate, Opa_{CEA-B} to CEA) (5 points).



B. The graph on the right is a measure of the number of exfoliating cells per unit of area after infection of either WT mice or CEA transgenic mice with no bacteria (uninfected), Opa_{CEA} or Opa_{HSPG} Ngo bacteria. After summarizing the results of this graph, explain in as much detail as possible what the effect is of Opa_{CEA} binding to CEA in the context of this infection. (5 points)



C. One question arising from the previous data stems from the fact that CEA is expressed apically in epithelia and the phenotype of Ngo transfection appears to be on the attachment of cells. One candidate gene to potentially explain this conundrum is CD105, a basally expressed protein that is overexpressed upon their interaction with bacteria on CEA expressing cells. Scientists performed similar experiments but with mice transduced with either an empty vector carrying just GFP or a vector carrying GFP and a small hairpin RNA targeting CD105 (shCD105). Interpret the graph below and explain whether CD105 is indeed the missing link and summarize your understanding of these experiments by using short sentences that list the different molecular factors. (6 points)



2015 MCD Reading List:

[1] Saqcena, M., Mukhopadhyay, S., Hosny, C., Alhamed, A., Chatterjee, A., and Foster, D. A. (2014) Blocking anaplerotic entry of glutamine into the TCA cycle sensitizes K-Ras mutant cancer cells to cytotoxic drugs, *Oncogene*.

[2] Saqcena, M., Menon, D., Patel, D., Mukhopadhyay, S., Chow, V., and Foster, D. A. (2013) Amino acids and mTOR mediate distinct metabolic checkpoints in mammalian G1 cell cycle, *PloS one 8*, e74157.

[3] Heard, E., and Martienssen, R. A. (2014) Transgenerational epigenetic inheritance: myths and mechanisms, *Cell 157*, 95-109.

[4] Kerr, S. C., Ruppersburg, C. C., Francis, J. W., and Katz, D. J. (2014) SPR-5 and MET-2 function cooperatively to reestablish an epigenetic ground state during passage through the germ line, *Proceedings of the National Academy of Sciences of the United States of America 111*, 9509-9514.

[5] Lee, M. J., Lee, J. H., and Rubinsztein, D. C. (2013) Tau degradation: the ubiquitin-proteasome system versus the autophagy-lysosome system, *Prog Neurobiol 105*, 49-59.

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[14] Hamza, I., and Dailey, H. A. (2012) One ring to rule them all: trafficking of heme and heme synthesis intermediates in the metazoans, *Biochim Biophys Acta 1823*, 1617-1632.

[15] Poole, E., Elton, D., Medcalf, L., and Digard, P. (2004) Functional domains of the influenza A virus PB2 protein: identification of NP- and PB1-binding sites, *Virology 321*, 120-133.

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[18] Zhu, Y., Regunath, K., Jacq, X., and Prives, C. (2013) Cisplatin causes cell death via TAB1 regulation of p53/MDM2/MDMX circuitry, *Genes & development 27*, 1739-1751.
[19] Muenzner, P., Bachmann, V., Zimmermann, W., Hentschel, J., and Hauck, C. R. (2010) Human-restricted bacterial pathogens block shedding of epithelial cells by stimulating integrin activation, *Science 329*, 1197-1201.
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