PhD Program in Biology
MCD First Exam Fall 2017

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 and complete sentences. This section requires a general essay that must answer the questions below. Each question is worth 15 points.

Question 1:
A. What is Unfolded Protein Response (UPR)? What triggers the UPR? Explain how this pathway is regulated at the transcriptional level. Explain what are potential consequences of the UPR response. Provide one example of the UPR associated with a pathological condition. (5 points)

B. Briefly explain one of the three molecular pathways/branches of the UPR. (5 points)

C. What is the role of autophagy in the UPR? Briefly discuss the molecular mechanism involved. (5 points)

References:
Walter and Ron, 2011. The Unfolded Protein Response: from stress pathway to homeotic regulation. Science 334, 1081-6.

Fumagalli et al., 2016. Translocon component Sec62 acts in endoplasmic reticulum turnover during stress recovery. Nat Cell Biol 18(11), p. 1173-1184.

Question 2:
The human fertilized egg gives rise to hundreds of distinct cell types in the adult.

A. Describe two methods for reversing the progressive restriction of cell fate that accompanies this process of differentiation. (7 points)

B. How might cells in which developmental potential has been reset, when taken from a diseased individual, be clinically valuable in vivo? Be specific and include examples. (4 points)

C. How might they be valuable in vitro? Be specific and include examples. (4 points)

References:
Tachibana, M. et al., 2013. Human embryonic stem cells derived by somatic cell nuclear transfer. Cell 153, p. 1228-1238.

Ebert et al., 2009. Independent pluripotent stem cells from a spinal muscular atrophy patient. Nature 457, p. 277-280.

Question 3:
Question about non-genetic transgenerational inheritance (epigenetics).

A. Describe a mechanism by which a gene activity can be altered in the absence of DNA changes in sequence? (5 points)

B. Contrast the roles of histone modification and non-coding RNA in epigenetic inheritance. (5 points)

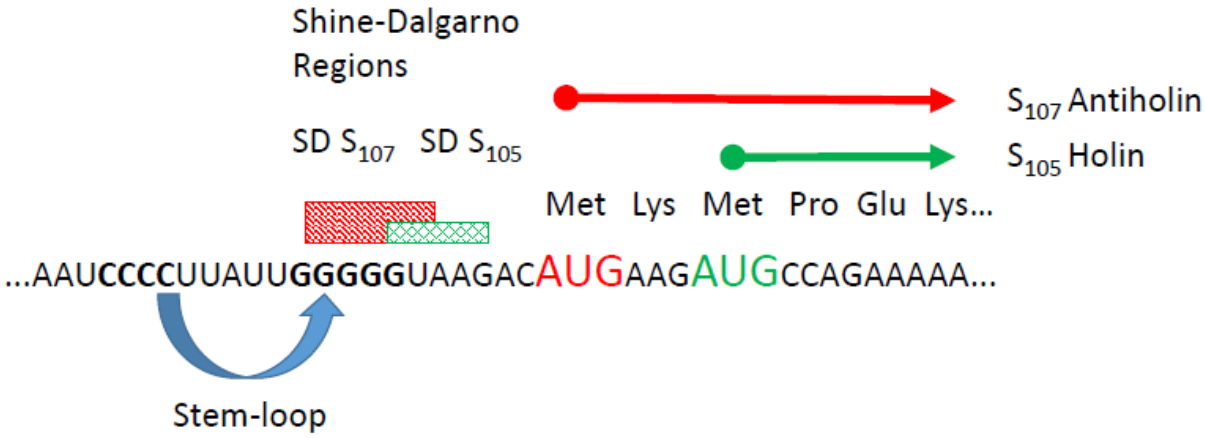
C. What techniques would you use to distinguish whether an inheritable altered gene activity is due to genetic mutation or it is due to an epigenetic effect. (5 points)

References:
C. D. Allis and T. Jeuwein, 2016. Molecular hallmarks of epigenetic control. Nature Reviews Genetics 17, p. 487-500.

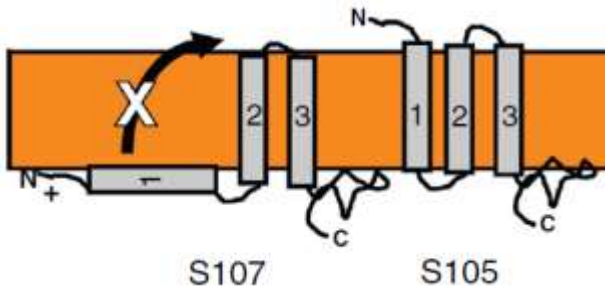
Stuwe, E. et al., 2014. Small but sturdy: small RNAs in cellular memory and epigenetics. Genes & Development 28: p. 423-431.

Question 4:

A. Explain the gene expression regulatory mechanisms that lead to the differential expression of the two bacteriophage λ lysis proteins, holin and antiholin. Use the diagrams below as appropriate. (8 points)



B. What evidence supports the hypothesis that antiholin acts as a specific inhibitor of holin? What mechanism is hypothesized to explain antiholin's inhibitory activity despite **being 98%** identical in amino acid sequence? What experiments support this hypothesized mechanism? Please explain the diagram in an essay, using full sentences. (7 points)



References:

Gründling, A., D.L. Smith, U. Bläsi, & R. Young. 2000. Dimerization between the holin and holin inhibitor of phage λ. *Journal of Bacteriology*, 182 (21), 6075–6081.

Chang, C.Y., Kiebang N., & Young R. 1995. S gene expression and the timing of lysis by bacteriophage λ. *Journal of Bacteriology*, 177(11), p. 3283-3294.

Question 5:
Taxanes, such as paclitaxel (Taxol), stabilize microtubules and prevent their depolymerization. They are commonly used as chemotherapeutic agents.

A. Describe the cellular consequences of paclitaxel treatment. Is there a dependency on p53 status, and what are the implications for the use of paclitaxel? (6 points)

B. Give two reasons (out of the three discussed by Bonezzi and colleagues) for suspecting that the molecular mechanisms underlying the inhibition of motility and mitosis are likely to be different. (2 points)

C. In addition to blocking cell proliferation, taxanes inhibit cell motility. What is known about the mechanism by which taxanes inhibit motility, according to Bonezzi and colleagues? Summarize their evidence. (4 points)

D. Cancer cells can evolve resistance to paclitaxel. New chemotherapeutics are being developed to target KIF11 (kinesin-5). KIF11 is a microtubule motor protein that functions during mitosis to position the chromosomes and establishing the bipolar spindle. Compare the possible effects of inhibiting KIF11 to the effect of taxanes. (3 points)

References:
Wang et al., 2000. Paclitaxel-Induced Cell Death: Where the cell cycle and apoptosis come together. *Cancer* 88, p. 2619–28.

K. Bonezzi et al., 2012. Inhibition of SIRT2 potentiates the anti-motility activity of Taxanes. *Neoplasia* Vol 14:9 p.846-854.

Question 6:
It has been speculated that the most commonly dysregulated signals in human cancer cell are those that lead to elevated activity of mTOR. mTOR promotes cell cycle progression and survival of cancer cells. As a consequence, there has been much interest in targeting mTOR therapeutically in many human cancers. However, there are important issues regarding the dose of rapamycin needed arrest cell cycle progression and to induce apoptosis.

A. Explain why the high doses of rapamycin needed to inhibit 4E-BP1 phosphorylation are not due to off-target effects. (5 points)

B. Suggest ways that rapamycin could be less toxic and still suppress 4E-BP1 phosphorylation. (5 points)

C. Explain the differential effects of high and low doses of rapamycin needed to cause cell cycle arrest. (5 points)

References:
Chatterjee A, Mukhopadhyay S, Tung K, Patel D, and **Foster DA**., 2015. Rapamycin employs TGF-β and Rb pathways to cause cell cycle arrest. *Cancer Lett.* 360, 134-140.

Mukhopadhyay S, Frias MA, Chatterjee A, Yellen P, and **Foster DA**, 2016. The enigma of rapamycin dosage. *Mol Cancer Ther.* 15, 347-353.

Part 2A - Experimental Design: This section of the exam is worth 20% of the total grade of the exam. Answer either question 7 or question 8- DO NOT ANSWER BOTH.

Question 7:
The transition to multi-cellularity has occurred numerous times in all domains of life, but its initial steps are poorly understood. The working hypothesis for you is: "In eukaryotes there are only a few gene products responsible for the initial steps for gaining multi-cellularity."
A. (12 points) Design an experiment that tests the given hypothesis, using three different green algae:

1. *Chlamydomonas reinhardtii*
2. *Gonium pectorale*
3. *Volvox carteri*

The experimental design should be laid out in detail with a rationale on why certain experiments should be performed. Explain with justification which of the three algal species would be your reference. Based on the cell cycle pathway evolution from the paper Hanschen et al. 2016, what would be the primary question that you would address? What would be your expectations for results and why?

B. (8 points) Taking advantage of genome sequencing technologies and comparative genomics in regards to cell cycle elements, describe an approach to find more genes responsible for the 12 steps (Hanschen et al. 2016) leading to multi-cellularity including other related green algae within the Volvocales. Based on the papers from Hanschen et al. 2016 and Umen 2014, what kind of species would you choose for your proposed work? Besides comparative genomics on known cell cycle genes, what other alternative experimental techniques could be used to identify new genes involved in multi-cellularity? For this alternate approach remember the differences in morphological phenotypes among the different species within the Volvocales.

References:
Hanschen, E. R. et al., 2016. The *Gonium pectorale* genome demonstrates co-option of cell cycle regulation during the evolution of multi-cellularity. *Nature Communications*, 7 , art. no. 11370

Umen J.G.V, 2014. Green Algae and the Origins of Multicellularity in the Plant Kingdom. *Cold Spring Harbor Perspectives in Biology*, 6 (11) p. 27.

Question 8.
Self-tolerance is a critical aspect of a properly functioning adaptive immune system. During development of T lymphocytes in the thymus randomly rearranged T cell antigen receptors (TCRs) are tested against an array of self-proteins presented on thymic epithelial cells and dendritic cells (DCs). Thymocytes bearing receptors that bind with high affinity to self are either induced to become regulatory T cells or are removed from the repertoire to prevent the development of autoreactive T cells that would ultimately lead to autoimmune diseases. The process by which a mirror of the peripheral self is expressed in the thymus and presented to the developing T cells is called promiscuous gene expression or tissue restricted antigen expression and is dependent on the functions of the auto immune regulator Aire and the more classic transcription factor Fezf2, that drive the expression of a mostly non-overlapping set of tissue restricted antigens (TRAs) in medullary thymic epithelial cells. The parallel RANK/CD40 and lymphotoxin beta receptor (LT βR) signaling pathways were shown to regulate the expression of Aire and Fezf2, respectively.

A. (10 points) Given that Aire KO and Fezf2 KO mouse models exist, design an experiment to identify the genes whose expression is directly controlled by Aire and Fezf2. How would you show that Fezf2 controls tissue restricted antigens expression independently of Aire? Include experiments that will show that Fezf2 binds directly to the promotors of Fezf2 dependent TRAs but not Aire dependent TRAs. Include a discussion of the appropriate controls and expected results for your experiments.

B. (10 points) Design an experiment using conditional mouse models to show that loss of either Aire or Fezf2 specifically in thymic epithelial cells leads to the development of autoimmune disease. Include experiments to confirm that deletion of either Aire or Fezf2 did not impact the development of thymic epithelial cells or the expression of the alternative protein within the thymus.

References:
Takaba et al., 2015. Fezf2 Orchestratesa Thymic Program of Self Antigen Expression for Immune Tolerance. *Cell* 163, p. 975-987.

Liston et al., 2003. Aire regulates negative selection of organ-specific T cells. Nature Immunology 4(4), p. 350-354.

PART 2B Data Interpretation: This section of the exam is worth 20% of the total grade of the exam. Answer either question 9 or question 10- DO NOT ANSWER BOTH QUESTIONS.

Question 9:

A healthy human gut microbiome is comprised of a great diversity of bacteria and other microorganisms. The gut microbiota promote health through modulating immune responses, competing with and preventing colonization by potentially pathogenic bacteria, providing beneficial compounds resulting from the fermentation of complex dietary fiber, and other important functions. Not surprisingly, the gut microbial community is able to metabolize orally ingested drugs and this can have significant effects on the efficacy and toxicity of a wide range of drugs prescribed to patients. One of the best-studied examples of this is the conversion of the cardiac glycoside, digoxin, to the inactive dihydrodigoxin. Reports from the early 1980s provided evidence that a subset of patients taking broad spectrum oral antibiotics and digoxin showed markedly increased serum levels of digoxin, suggesting the possibility that anaerobic gut bacteria were responsible for the conversion of the active digoxin into the inactive dihydrodigoxin.

A. (10 points) As a result of the work in the early 1980s, researchers were able to show that the anaerobic gut bacterium, *Eggerthella lenta*, was the species responsible for the inactivation of digoxin in the human gut. This success prompted physicians to test for the presence and abundance of *E. lenta* in the stool of patients prescribed digoxin in the belief that higher levels of *E. lenta* would result in less digoxin (and more of the inactive dihydrodigoxin) detected in the patient serum. It was hoped that the therapeutic dose of digoxin required could be achieved by raising the amount of digoxin prescribed in the case of patients with high levels of *E. lenta*. However, research showed little inverse correlation between the abundance of *E. lenta* in a patient's stool and reduced levels of serum digoxin. What factors might explain these unexpected results?

B. (4 points) Examining figure 3 from Haiser et al. (*Science*, 2013), what is the effect of increased dietary protein on the levels of digoxin in serum and urine?

C. (6 points) What explains the difference seen between the two strains of *E. lenta* in Figure 3C and 3D? Briefly describe the most likely mechanism, based on the data from Haiser et al., explaining the changes in level of digoxin in serum and urine when comparing the 0% and 20% dietary protein feeding protocol?

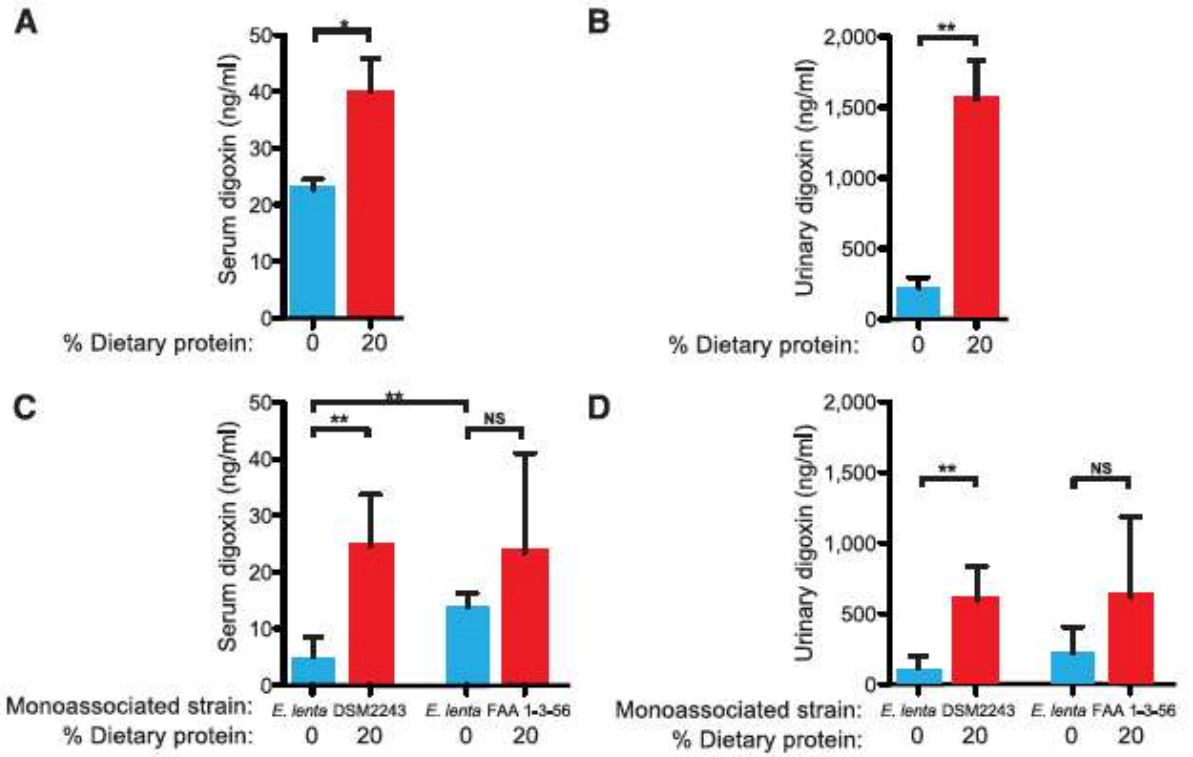


Fig. 3. Dietary protein blocks the inactivation of digoxin. Serum (**A**) and urinary (**B**) digoxin levels from the type strain experiment. Fecal digoxin levels showed a consistent trend: the mean area under the curve was 6.226 ng digoxin per hour per ml in germ-free mice, 3.576 for mice fed the 0% protein diet, and 6.364 for mice fed the 20% protein diet. Serum (**C**) and urinary (**D**) digoxin levels from each group. Digoxin levels were quantified by enzyme-linked immunosorbent assay (ELISA) (*7*). Values are means ± SEM. Asterisks indicate statistical significance by Student's *t* test (*$P < 0.05$; **$P < 0.01$). $n = 4$ to 5 mice per group. NS, not significant.

References:

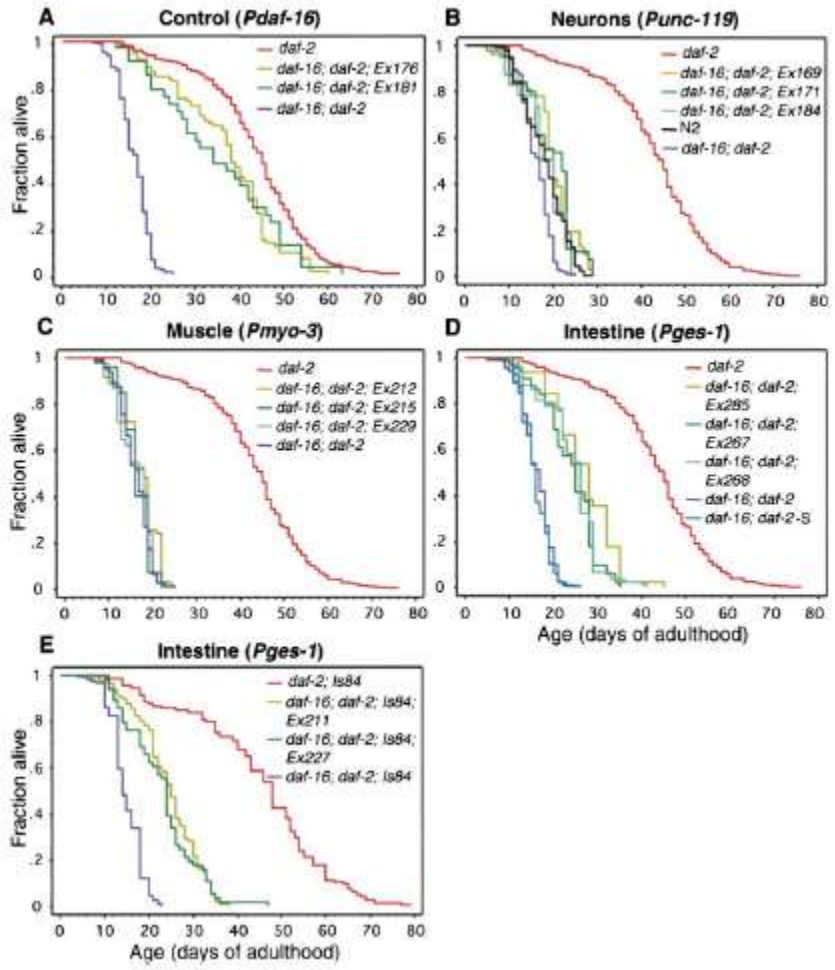
Haiser et al., 2013. Predicting and Manipulating Cardiac Drug Inactivation by the Human Gut Bacterium *Eggerthella lenta. Science* 341: p. 295-298.

Haiser et al., 2014. Mechanistic insight into digoxin inactivation by Eggerthella lenta augments our understanding of its pharmacokinetics. *Gut Microbes* 5:2, p. 233-238.

Question 10.

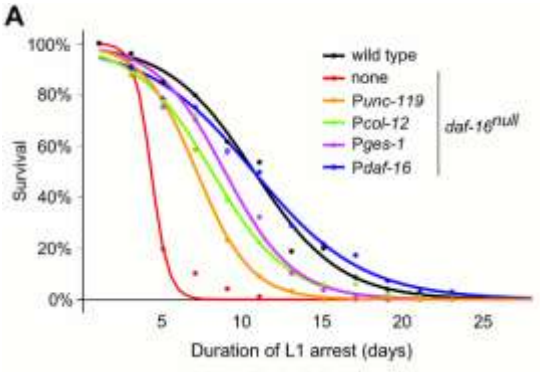
Insulin/IGF-1-like signaling (IIS) is a conserved mechanism by which animals respond to dietary status. In *C. elegans*, the IIS pathway mediates several such responses, including dauer arrest, longevity, and fat accumulation. Several *C. elegans* insulin-like ligands exist, but all are thought to act through the sole insulin receptor, DAF-2. The insulin receptor then acts through a downstream transcription factor, DAF-16, a member of the FoxO family of transcription factors. DAF-2/InsR activity represses the function of DAF-16/FoxO. Genetically, this relationship is revealed as epistasis: *daf-16* loss-of-function suppresses the phenotypes of *daf-2* mutants and *daf-2;daf-16* double mutants show the *daf-16* single mutant phenotype.

A strength of the *C. elegans* system is the ability to dissect the tissue-specificity of a signaling pathway in its physiological context. One way to do this is to express a gene product in specific tissues in an otherwise mutant genetic background using well-characterized tissue-specific promoters. Taking this approach, the tissue-specificity of DAF-16 function was determined.



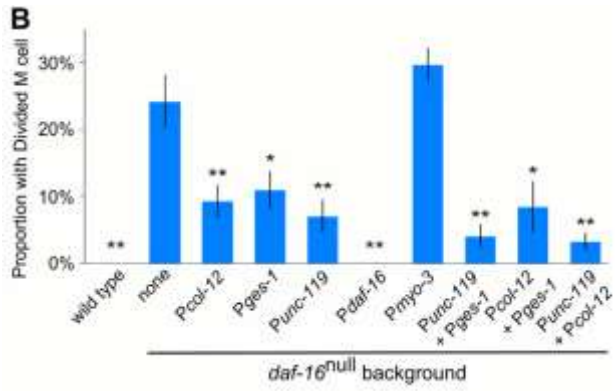
A. (5 points) The data shown here were obtained in an experiment to test the tissue-specificity of DAF-16 in lifespan regulation. What do you conclude from this experiment?

B. (5 points) If *C. elegans* larvae are hatched in the absence of food, the first-larval stage (L1) animals will halt development. This L1 arrest is also regulated by IIS. In an experiment similar to the one above, the role of DAF-16 in specific tissues to promote survival during L1 arrest was determined. What do you conclude from this experiment?



Promoter	Expression
unc-119	neurons
col-12	skin
ges-1	intestine
daf-16	many tissues

C. (5 points) When *C. elegans* enter L1 arrest, cell division is blocked until development can resume. One such cell, which does not divide in arrested larvae, is the M cell, the precursor to muscle. In contrast, in *daf-16* mutant animals undergoing L1 arrest, the M cell is seen to divide inappropriately in 25% of animals (see figure below). To determine where DAF-16 acts to prevent M cell division during L1 arrest, animals with tissue-specific *daf-16* expression were again analyzed. What do you conclude from this experiment?



Promoter	Expression
unc-119	neurons
col-12	skin
ges-1	intestine
daf-16	many tissues
myo-3	muscle

D. (5 points) In general, does DAF-16/FoxO act cell autonomously or nonautonomously? Briefly speculate about how a transcription factor can function in this way.

References:

Libina, Berman and Kenyon, 2003. Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. Cell 115: 489-502.

Praitis and Maduro, 2011. Transgenesis in *C. elegans*. Methods in Cell Biology 106: 161-185.