Q1: A) How do morphogens determine the patterning of tissues during development?

B) How does sonic hedgehog (SHH) signaling result in distinct and spatially organized cell fates in the ventral spinal cord? (5 points)

C) Briefly contrast the Shh signaling pathway that result in chemotaxis of neuronal growth cones to Shh signaling that leads to cell fate determination. (5 points)

References:


Q2:

Two classic papers on DNA replication were provided covering 15 years of research on the discovery of the duplex structure for DNA. For each of the papers that you read, summarize the key points and compare it to what modern molecular biology currently teaches – put it in perspective.

A) Describe the mechanism of replication based on Meselson-Stahl experiment- Review the methods, results and conclusions from this research paper.

B) Describe the mechanism of replication based on Okazaki et al. experiment- Review the methods, results and conclusions from this research paper.

These are the elements of a complete answer in compact form. A good answer to the question would include all elements listed, expanded in a coherent and detailed way. Illustrations would be desirable but may not be practical, depending on how the exam is administered.

References:


Q3: INNATE IMMUNITY QUESTIONS

The innate immune system provides the host's first line of defense against microbial pathogens, acting as an early antimicrobial protective mechanism by, in part, inducing an inflammatory response comprised of a cascade of events leading to pathogen killing and eventual tissue repair. The innate immune system is also responsible for the early events of sterile inflammation occurring in the absence of pathogens. Recently studies suggest that the innate immune system may play a role in neurodegenerative diseases such as Alzheimer’s disease.

a. What is a PAMP? (1 point)

b. Describe the response of monocytes/macrophages to a PAMP that activates via TLR4. (1 point)

c. How many signaling pathways are there for TLR4? (2 points)
d. Describe the different molecules required for the signaling pathways, the products produced and indicate the roles of the products in inflammation. (4 points)

e. What evidence suggests that TLRs may play a role in Alzheimer’s disease? (5 points)

f. Discuss the implications of these results in AD. (2 points)

References:


Q4:
DNA Double Strand break (DSB) repair is critical for genomic stability. How two different types of DSB repair exist, with one resulting in an error free pathway and the other, being error prone. Please write an essay about these pathways using the prompts provided below to help you articulate the necessary details.

A) Describe (and name) the two different pathways that are used for DSB repair. Make sure to include how these pathways impact genome stability or genomic instability. Follow this up by comparing and contrasting the molecular mechanisms used for each pathway and the proteins that regulate the processes.

B) What protein is able to regulate the “repair choice” and how does this protein function to shift repair from one pathway to the other?

C) CEP-1 was recently found to be involved in DSB repair required to ensure meiotic fidelity. Describe the well-known function of CEP-1 and describe how this DSB function was discovered and how it is different from the well-known textbook CEP-1 pathway.

References:


Q5:
“`The City that Never Sleeps`” is one of the nick names of New York City. Yet, according to a recent story in the New York times, it should instead be “The City That Ought to Sleep”. Sleep represents a typical circadian body rhythm. Build an essay around the following questions to demonstrate your understanding of the mechanism underlying sleep/wake circadian activity.

A) How were the first mutations with perturbation in rhythmic behavior identified? Name the mutagen used and explain how it affects DNA structure.

B) What was the genetic scheme for selection of mutants?

C) How many mutations were identified? Briefly describe the assays used. How many genetic loci did these mutations represent and how was this information determined? What were the phenotypes of the mutants identified and what was the molecular basis of these phenotypes?
D) What were the types of phenotypes and the nature of alleles identified in the study? (10 points)

E) Identify two discrete post-translational mechanisms by which the “Molecular Feedback Loop” model explains individual proteins in this molecular model are regulated. (5 points)

References:


Q6:
For most cells the linear distance of their genome, put end to end, vastly exceeds any of their linear dimensions. This fact indicates that DNA has to be condensed multiple times to fit inside a cell. On the other end, most understanding of the regulation of genes involves molecular interactions with the DNA double helix. This poses a conundrum.

A) Use the physical dimensions of DNA to estimate the length of the genome from the cell of your choice. Given the size of a cell, illustrate quantitatively how much compaction of DNA is necessary and how this is feasible. You might want to compare the volume of DNA, to the volume of the structure which contains the DNA (5 points).

B) Based on your general knowledge, explain the mechanisms that enable the DNA to be so compacted. You will start with compaction mechanisms at scales close to the scale of the DNA double helix and then move your way up. Please identify the molecular players involved. (4 points).

C) Illustrate how condensin complexes enable access to the double helix of DNA among all the compacted chromatin. (6 points).

References:


Part 2A: Experimental Design
Q7
Chimeric Antigen Receptors (CARs) are currently being used to reprogram T cells to recognize and kill B cell lymphomas (Morgan and Boyerinas 2016). The generation of these genetically engineered T cells requires the assembly of genetically modified retroviruses. (20 points)

A) CARSrUS, Inc. has hired you to design a new treatment for melanoma using CAR T cell therapy. Their CAR (3B2) specifically recognizes a protein on the surface of melanoma cells, which will allow the engineered T cells to kill the melanoma cells. CARSrUS also wants to identify the T cells that express the CAR by labelling the infected cells with GFP; however, to avoid disrupting the function of the CAR, they do not want to create a chimeric 3B2-GFP fusion protein. Explain how you will assemble a modified retrovirus that will reprogram T cells to recognize melanoma and simultaneously mark the T cells with GFP. Specify what genes will be included in the modified retrovirus genome and what genes will be used to assemble the virus in the packaging cells. (10 points)
B) The scientists at CARsRuS are concerned that your modified retrovirus could activate a gene (MYC) in the T cells when the modified retroviral genome integrates into the T cell genome. Describe two experiments that you could perform to determine whether or not the MYC gene is disrupted by the integration of the modified retroviral genome into the T cell genome. (10 points)

References:


Part 2A: Experimental Design
Q8 (Total: 20 pts)

Question on CRISPR
A) (4 pts) In a few sentences, describe your overall understanding of CRISPR-mediated bacterial immunity. How does CRISPR immunity discriminate between self and non-self?

B) Your laboratory is studying a bacterial species that encodes a poorly characterized CRISPR/Cas locus. Your lab mate has isolated 5 virulent bacteriophage isolates from the East River that plate well (i.e. grow) in a CRISPR- strain of this bacterium. Most of the genes in the genomes are similar between the phages and identifiable - i.e. genes for capsids, replicases, etc. Given that some CRISPR/Cas systems can target foreign DNA or RNA, your first aim is to establish the nature of the nucleic acid being targeted by the crRNA (CRISPR RNA) in this bacterium. You find that all 5 phage isolates plate well in a CRISPR- strain, but only one plates well in a CRISPR+ strain. When you sequence the spacer locus in your bacterium you find that one of the spacers is identical to the capsid gene sequence in all 5 phage isolates.

i. (8 pts) Devise a genetic experiment that would allow you to figure out the nature of the crRNA mediated target in this system.

C) (8 pts) Recall that one phage isolate can plate well in a CRISPR+ strain of the bacterium. What do you predict could be happening here and how would you test your prediction?

References:


For Part 2B: Data interpretation
Q9:

You are studying the regulation of three genes (gene “A” and gene “B” and gene “C”). Based on prior genetic data, it is known that soluble Factor “D” is required for the activation of these three genes. Thus, it is hypothesized that these genes transcriptional activity will be responsive to cell stimulation with factor “D”.

Chromatin immuno-precipitation is employed to study key epigenetic marks present at the three gene loci (A, B and C) in the presence and absence of Factor D treatment. The following data are obtained:

<table>
<thead>
<tr>
<th>Cells BEFORE Factor D treatment</th>
<th>Cells AFTER Factor D treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>H3K4me3</td>
</tr>
<tr>
<td>A</td>
<td>negative</td>
</tr>
<tr>
<td>B</td>
<td>positive</td>
</tr>
</tbody>
</table>
C negative negative C positive negative

Negative means not detected at the gene locus. Positive means readily detected at the gene locus.

Based on your interpretation of the above data, please answer the following:

i. Which of the three genes above would you expect to be producing complete mRNA transcripts in the presence of Factor D? Please briefly explain your reasoning.

ii. What hypotheses would you propose to explain the distinct role of Factor D in the activation mechanisms of each of the three genes (gene A, gene B and gene C)?

iii. Please describe what other molecular evidence you would expect to find at the gene A, gene B and gene C locus that would further support your initial hypotheses (described in part ii) derived from your interpretation of the above data.

References:


Part 2B: Data interpretation
Q10

Phosphorylation of the Ser-15 site of p53 is thought to be one of the initiating phosphorylation events following cellular stress that stimulates subsequent modification and stabilization of the p53 protein. In this study, researchers examined the roles of p53, RPA and ATR and their relationship to transcriptional stress.

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construct expressing a dominant-negative Nup160 protein and 6 h later the cells were fixed and stained. In all figures, the white arrows indicate microinjected cells.

Abbreviations: IgG, rabbit IgG; DAPI, nuclear stain; TAP, Tip-Associated Protein (also known as NXF1 and MEX67); EGFP, Enhanced green fluorescent protein; Nup160, nucleoporin protein Nup160.

Summarize the results in the figures and address the following questions in your answers:

A. What can you determine about the localization and the status of poly(A) RNA in figure A and explain how you made these determinations. (4 points)

B. What is the purpose of the CMV-EGFP-Luc construct used in (B) and what can be determined by examining the cells microinjected with this construct? (2 points)

C. Explain why the researchers used antibodies to TAP in these assays. (1 point)

D. What can you conclude about p53 and its phosphorylation from these assays and which figure(s) allowed you reach your conclusion(s)? In your explanation, include a description of figure D and how it relates to figure F. What is the difference between these results and the results shown in figures C and E? What general conclusions can you reach from these four figures? (8 points)

E. What does the experiment using cells expressing a dominant-negative Nup160 (dnNup160) protein show and why did they perform this experiment? Were there additional experiments the authors might have performed to support their argument? (4 points)

F. What conclusions can you draw about p53 and mRNA from this set of figures? (1 point)

References: