Instructions:

The following pages contain twelve questions. You must answer ten out of the twelve questions. Any one question may ask for more than one response, so please read each question carefully for specific instructions pertaining to that question. Answers should be written neatly in ink, since the exams will be photocopied prior to grading. Paper will be provided. Write only on one side of each piece of paper. Please do not write to the extreme edge of the paper as it makes photocopying difficult/impossible. Make sure the graders can read your answers clearly. Please remember as you answer the questions that you have ~45 minutes per question. This means that we expect in depth answers. Use diagrams to illustrate your answer wherever possible. Although each question is worth the same number of points, not all questions will require the same amount of time. Allocate your time wisely.

We recommend that you first make an outline of your answer, rather than just making up your answer as you write. Some questions ask you to propose experiments. Choose the most direct and realistic approaches and explain your experimental rationale as clearly as possible. Be sure to include alternate hypotheses, controls, expected outcomes and possible problems and solutions.

Do NOT put your name on any of the question or answer sheets. So that the exam will remain anonymous, label your pages with the question number (e.g., Question 1) and with a coded name, using the code distributed by Susan.

PLEASE MAKE SURE THAT ALL PAGES ARE NUMBERED and IDENTIFIED!!!!.

This is a closed book exam. Absolutely no discussion will be allowed between the students while the exam is in progress. You will be held to an honor code by agreeing to not receive or give aid on this exam.

You will have from 8:00 a.m. until 5:00 p.m. each day to finish each part of the exam. Lunch will be available from 12:00-1:00. No exam materials can leave the room with you during lunchtime or any other time. The exams will be collected no later than 5:00 p.m. sharp.

If you have questions please contact David Pallas (4125 RRC) 7-5620.
1. A single *E. coli* cell is added to a medium containing $^{15}$NH$_4$Cl and allowed to divide for many generations. The resultant cells are then shifted to a medium containing $^{14}$NH$_4$Cl. DNA is isolated from cells grown for one generation in $^{14}$NH$_4$Cl, denatured by heating and subsequently centrifuged in a CsCl density gradient. Two bands are obtained. The denser band is isolated and analyzed. Answer the following questions and explain your answers.

a) (2 points) Does the isolated fraction (denser band) contain DNA polymers that are complementary to each other or DNA only polymers that are identical in nucleotide sequence?

b) (2 points) Were all of the DNA polymers in the isolated fraction (denser band) synthesized in the same generation?

B. Consider the amino acid sequence below, which represents the amino terminus of a protein synthesized in an unstudied organism for which you have no genomic or cDNA sequence data. Which of the following statements is/are true and which is/are false? Justify your answers.

\[
\text{NH}_2-\text{MET-LEU-ALA-TRP-GLY-ARG-COOH}
\]

a) (1.5 points) The peptide sequence provides sufficient information to determine whether it was synthesized in a prokaryote or a eukaryote.

b) (1.5 points) The peptide sequence provides sufficient information to determine the sequence of the portion of mRNA that encodes it.

c) (1.5 points) The peptide sequence provides sufficient information to determine the relative order in which each amino acid was covalently added to the growing peptide chain during synthesis.

d) (1.5 points) The peptide sequence provides sufficient information to determine the location of a splice site in the RNA that encodes it.

2. The gene JUMP was cloned into an inducible vector and introduced into cultured NIH3T3 cells. Microarrays were performed on uninduced cells and on cells in which JUMP expression had been induced. The results of this DNA microarray experiment suggest that the gene JUMP activates expression of the gene HOW HIGH.

A) (2 points) Describe one method that you could use to confirm your microarray result?

B) (2 points) Would (an) additional experiment(s) be necessary to conclude that JUMP increases the protein level of HOWHIGH? If so, describe, including controls. Assume you have the necessary reagents.

C) (3 points) Describe two additional experimental approaches (other than an inducible promoter and microarray) you would use to perturb the levels of JUMP to determine whether it regulates the level of HOWHIGH. Be sure to include controls.

Briefly (ie, a couple sentences) describe an additional experiment you would perform to support the link between JUMP and HOWHIGH if

D) (1 point) -JUMP were a known receptor? (also state of the rationale for your experiment)

E) (1 point) -JUMP were a kinase? (also state of the rationale for your experiment)

F) (1 point) -JUMP were a transcription factor? (also state of the rationale for your experiment)

Assume you already have the necessary reagents that are specific to genes JUMP and HOWHIGH. Be sure to include control experiments.
3. This year’s BCMB Symposium was on Germ Cells, with a range of speakers covering a number of topics relevant, but not restricted to, germ cell biology. These included the regulation of germ cell “differentiation” in adult gonads, such as regulation of exit from mitosis and entry into meiosis, as well as engagement of gamete-specific transcription programs. Other topics ranged from factors and pathways regulating germ cell migrations in mid-stage embryos, to maintenance of germ cell identity in the early embryo. The final talk dealt with the switch from germline to soma identity in early embryonic blastomeres—how somatic lineages “shake off” their oocyte past to engage in somatic developmental pathways.

A) (5 points) Discuss what is meant by the “germline/soma” distinction. Please define and discuss three fundamental differences between these two basic types of cell lineages.

B) (3 points) Pick one of the topics discussed by one of the speakers, describe the problem or question that was addressed, and suggest an experimental approach that could be used to determine the mechanisms involved. Include some experimental detail in your answer. You are free to use the experimental approach chosen by the speaker or to design a different approach.

C) (2 points) Discuss how the question or topic you chose in ‘B’ is also a question relevant to somatic lineages.

4) Step back into the past ~10 years. You are interested in understanding how your favorite protein is targeted into the nucleus. You have determined that a specific amino acid sequence within your protein of interest is essential for its nuclear localization. You term this signal the Nuclear Localization Signal (NLS). In fact, you have identified a variant of this protein that is not targeted to the nucleus. This variant contains a single point mutation in the NLS you have identified. Although you have defined the NLS, you do not know how your protein is targeted to the nucleus for import. A) How could you identify a soluble transport receptor responsible for targeting your protein of interest to the nuclear pore complex? Be sure to include controls to demonstrate that the protein you identify is indeed the receptor that recognizes the functional NLS in your protein of interest (5 points).

Now step back into the present. B) Once you identify the putative receptor, how could you test whether it is actually the functional import receptor for your protein of interest (3 points)? C) Based on what is know about the nuclear transport process today (i.e., not 10 years ago), what type of protein might you expect to identify in your studies (1 point)? D) Is there an experimental strategy that you could incorporate into the modern version of this experiment to demonstrate that putative receptor that you identified interacts with your cargo protein specifically as an import receptor (i.e., it does not bind like an export receptor nor is it simply a constitutive binding partner for your protein of interest) (1 point).

5. You are using a beta 1 receptor agonist drug (tachydarone) in an attempt to increase the heart rate of a patient to perform a type of stress test. Despite increasing doses well above the usual range (10mg/kg), there is no change in the heart rate. Then, you notice the patient is on a drug called amiodarone that is an inhibitor of the beta 1 receptor.

A) (2 points) What type of inhibition would most likely explain the ability of amiodarone to block the action of tachydarone even with increasing concentrations of tachydarone?
B) (2 points) What type of inhibition could be overcome?
C) (4 points) Describe the differences in these types of inhibition. Illustrate your answer.
D) (2 points) You give tachydarone to a second patient who is taking 10 mg/kg of a different beta 1 receptor inhibitor drug, metadarone. This patient’s heart rate increases when given 40 mg/kg, or four times the normal dose of tachydarone. Assuming that affinity is the only difference, what is the $K_d$ of tachydarone relative to metadarone?

6. Extracellular signals are known to stimulate reorientation and polarization of cells. These signals are transmitted by G protein-coupled receptors (e.g. fMLP receptors) and tyrosine kinase receptors (e.g. insulin receptors) to small GTPases of the Rho family. Downstream responses include recruitment of pleckstrin homology (PH) domain-containing proteins (e.g. Akt and PI-3K) to the plasma membrane and activation of actin polymerization. Based on the data presented in the following table, answer the questions posed below. Note that the cells used to obtain these results were HL-60 cells that are responsive to both fMLP and insulin. N17 constructs represent “dominant-negative” forms of the GTPases and V12 constructs represented activated forms.

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>PH-Akt-GFP recruitment</th>
<th>PH-Akt-GFP recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell number</td>
<td>% of cells</td>
</tr>
<tr>
<td>Control</td>
<td>fMLP</td>
<td>17/19</td>
</tr>
<tr>
<td>RacN17</td>
<td>fMLP</td>
<td>16/48</td>
</tr>
<tr>
<td>Cdc42N17</td>
<td>fMLP</td>
<td>10/11</td>
</tr>
<tr>
<td>WaspΔC</td>
<td>fMLP</td>
<td>13/15</td>
</tr>
<tr>
<td>Control</td>
<td>Insulin</td>
<td>13/16</td>
</tr>
<tr>
<td>RacN17</td>
<td>Insulin</td>
<td>25/30</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>0/20</td>
</tr>
<tr>
<td>RacV12</td>
<td>None</td>
<td>13/18</td>
</tr>
<tr>
<td>RacV12</td>
<td>Latrunculin B</td>
<td>3/19</td>
</tr>
<tr>
<td>Cdc42V12</td>
<td>None</td>
<td>0/12</td>
</tr>
</tbody>
</table>

A) (2 points) Briefly describe how you think these experiments were done. What do you think is being measured and how might that measurement have been done?

B) (4 points) What role is played by Rac in mediating stimulation by fMLP? What is the role of Cdc42? What is being stimulated?

C) (1 point) Why is Latrunculin B included? What do the Latrunculin results suggest?

D) (1.5 points) Briefly describe one other experiment that you might have included in this table and why you would have included it?

E) (1.5 points) Briefly describe one other experiment you might do to extend these results?
7. You have recently become interested in a particular gene. This gene encodes a protein that is highly conserved among all organisms. Your initial goal is to determine whether this gene is essential for viability in your favorite organism. A) (5 points) Choose a model organism and describe how you would test whether the gene is essential for life. In your description, include controls, how you would interpret your results, and one alternative approach. We’ll assume that there is sufficient rationale to merit testing the essential nature of this gene. B) (5 points) Assuming that you determine that the gene is essential for viability, briefly describe how could you begin to analyze the function of that essential gene in your model organism of choice.

8. You have recently identified a gene, obtained a cDNA, and produced an antibody to your protein. You have found out that the protein encoded by your gene is 100kDa and you have the entire sequence of the protein. You want to continue characterizing the protein product. Describe experiments to address the following questions. Be sure to include appropriate controls in your experiments and describe in some detail how you would obtain the reagents you propose to use.

A) Does the encoded protein exist as a soluble monomer, or as part of a stable multimer or complex? Describe two approaches, one that requires an immunoprecipitating antibody (3 points) and one that only requires a immunoblotting antibody (3 points).

B) (4 points) Assume the encoded protein exists as part of a complex. Describe one approach that you could use to determine the identity of the other macromolecules with which it associates?

9. Protein ZFD harbors a zinc finger domain that may potentially bind to DNA and/or RNA. You have just obtained a chemical named Zin that binds protein ZFD with high affinity in vitro and causes conformational alteration of the zinc finger domain. You treated cultured cells with Zin and found severe reduction of the mRNA encoded by gene X. Assuming that the complete sequence information about the gene encoding protein ZFD is available, please design experiments to answer the following questions.

A) (6 points) What are two alternative hypotheses that could explain the reduction of mRNA X upon treatment of cells with Zin? Consider hypotheses that are involve distinct mechanisms that could regulate the steady-state level of mRNA in a cell. Design an experiment to test each of your hypotheses.

B) (2 points) How could you determine whether elevated expression of protein ZFD is sufficient to cause elevated expression of mRNA X.

C) (2 points) Design an experiment to determine whether the zinc finger domain is necessary for the function of protein ZFD?

10. You are interested in the function of a particular protein. Your goal is to define the function of various amino acid residues within the protein using a site-directed mutagenesis approach. The charge from your advisor is to design the mutants.

A) For each of the following scenarios (i-iv) described below, provide a brief explanation of how the particular amino acid is likely to contribute to/mediate the proposed function. (1 point for each scenario)
B) Design specific amino acid changes to test the proposed function. For each scenario, please choose (a) an amino acid change that represents a conservative change and (b) one that represents a fairly drastic change. For each change (conservative and drastic), please provide the rationale behind your choices based on the predicted function/structure of that amino acid as described in the scenario. (1.5 points for each scenario)
NOTE: You do NOT need to describe the actual procedure for mutagenesis or functional analysis as they are beyond the scope of this question.

i) Your enzyme may be a cysteine protease. You want to test the hypothesis that cysteine 322 is required for catalytic activity.

ii) You have identified a highly conserved lysine in your protein of interest. You hypothesize that this residue might be critical for an interaction with a partner protein. Your initial thought is that the charge on the lysine may be required for the interaction.

iii) Your protein contains a highly conserved leucine residue that is present in a hydrophobic region in the protein interior.

iv) A conserved serine within your protein is critical for regulation of the protein activity.

11. You have purified an unusual protein from human brain, and now you want to clone the corresponding gene and cDNA from mouse. Remember that at the moment you only have a band on a gel.

   A) (3 points) How can you figure out if the mouse genome encodes a corresponding gene?
   B) (2 points) If the mouse genome does encode a corresponding gene, how could you clone it?
   C) (3 points) Once you have cloned the mouse genomic sequence, how can you obtain the full-length cDNA?
   D) (2 points) How will you know it is full-length?

12. A mutation in worms leads to a loss of all posterior muscle cells. You map the mutation to a small region on Chromosome III. Using the genome sequence, you identify several genes in the region that appear to encode transcription factors, one of which has a homologue in Drosophila (dHox-Z) that is known to be involved in posterior cell fate determination.

   A) (3.5 points) Describe an experiment that would demonstrate unambiguously that the phenotype you observe in your mutant worms is due to a mutation in the worm dHox-Z. Be complete!
   B) (3.5 points) Assume you have proven that the mutation is in this gene (wHox-Z). Describe a set of experiments in which you show that the expression pattern of this gene fits with its predicted function.
   C) (3 points) You suspect that the function of the gene is highly conserved between species. Describe an experiment to test this idea.
Day Two (The End is Near!) June 2\textsuperscript{nd}, 2004

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13. A) (2 points) Second messengers are important for cellular signaling. What are two features common to many second messengers that are important for their cellular function. Explain why they are important.

B) (8 points) Calcium is an important second messenger. Explain how intracellular calcium concentration is regulated. Among other things, include in your discussion how the cell maintains a different calcium concentration inside the cell compared to outside, at least one way that intracellular calcium concentration is raised transiently in response to receptor stimulation, at least two different KINDS of enzymes or cellular structures regulated by calcium, and how signaling by calcium is terminated once the receptor is turned off.

14) Below are the title and abstract of a recent paper. Although you don’t know exactly how the authors did the work, explain briefly but clearly how two of the three major findings (numbered) should have been obtained by these authors using the techniques and assays mentioned, or, if no technique is mentioned, by what you consider the best approach (5 points for each major finding). For each finding they report, describe how the experiments would be performed and what results they would need to obtain to prove their claims. Be sure to include controls. Do not give details of how you make reagents, just the experiments you would perform, controls, etc.

**PML is a direct p53 target that modulates p53 effector functions.**

de Stanchina E, Querido E, Narita M, Davuluri RV, Pandolfi PP, Ferbeyre G, Lowe SW. Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA.

The p53 tumor suppressor promotes cell cycle arrest or apoptosis in response to stress. Previous work suggests that the promyelocytic leukemia gene (PML) can act upstream of p53 to enhance transcription of p53 targets by recruiting p53 to nuclear bodies (NBs). 1) We show that PML is itself a p53 target gene that also acts downstream of p53 to potentiate its antiproliferative effects. Hence, p53 is required for PML induction in response to oncogenes and DNA damaging chemotherapeutics. 2) Furthermore, the PML gene contains p53 binding sites that confer p53 responsiveness to a heterologous reporter and can bind p53 in vitro and in vivo. 3) Finally, cells lacking PML show a reduced propensity to undergo senescence or apoptosis in response to p53 activation, despite the induction of several p53 target genes. These results identify an additional element of PML regulation and establish PML as a mediator of p53 tumor suppressor functions.

15. A) (4 points) Describe the two major pathways for protein degradation in a cell.

B) (2 points) You are studying a protein called ‘UNSTBL’. Design an experiment to determine which of these two pathways mediates the degradation of UNSTBL.

C) (2 points) Name two cellular processes that are regulated by protein degradation.

D) (2 points) What is an advantage to regulating these processes by degradation than by other mechanisms such as phosphorylation?
By definition, lipid molecules are hydrophobic causing them to aggregate in the presence of water.

A) (4 points) Explain what is meant by the critical micelle concentration (CMC). Explain how the CMC of a lipid molecule is related to its spontaneous transfer between intracellular membranes.

B) (3 points) How does the molecular shape of a lipid molecule determine the structure of the aggregate? Give two different examples of lipids with different molecular shapes that would form aggregates with different structures. Explain why.

C) (3 points) Give two examples of enzymes that are capable of altering the structure of phosphatidylcholine to change the molecular shape of the resulting molecule with the potential to alter the curvature of a bilayer membrane.

You have recently identified a gene by positional cloning, and northern blots demonstrate that the apparent exons in this region are transcribed and processed as part of what turns out to be a 3kb mRNA that is predominant in liver. Now you want to start characterizing the protein product that you assume is encoded by this large mRNA. Unfortunately, when you try to use a computer program to determine exons and predict the cDNA sequence, you get ambiguous results. Luckily, however, you are confident of one exon sequence that encodes the N-terminal 50 amino acids of the protein. What sorts of experiments would you propose to address the following questions? Be sure to include appropriate controls in your experiments and describe in some detail how you would obtain the reagents you propose to use (i.e., do not assume anything).

A) Since you don’t have a cDNA sequence, you have decided to make an antibody against the protein. Describe how would make the antibody? (2 points) Then describe how you would use the antibody to determine the molecular size of the encoded protein? (2 points)

B) (3 points) Describe one approach for determining the subcellular localization of your protein that utilizes your antibody.

C) (3 points) You finally obtain a cDNA clone for your protein. Describe another approach for determining the subcellular localization of your protein that does NOT utilize your antibody.
18. 

A  Constructs

<table>
<thead>
<tr>
<th>Extracellular</th>
<th>Cytoplasmic</th>
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<tbody>
<tr>
<td>E-cadherin</td>
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<tr>
<td>Desmoglein</td>
<td></td>
</tr>
<tr>
<td>E-cad-Dsg Chimera</td>
<td></td>
</tr>
<tr>
<td>Dsg-E-cad Chimera</td>
<td></td>
</tr>
</tbody>
</table>

B  Yeast Two Hybrid Results

<table>
<thead>
<tr>
<th>Desmoglein</th>
<th>E-cadherin</th>
<th>Plakoglobin</th>
<th>β-catenin</th>
<th>α-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>+</td>
<td>n</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Plakoglobin</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>β-catenin</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>α-catenin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

C  Co-IP Results

- E-cadherin
- Desmoglein
- E-cad-Dsg
- Dsg-E-cad

- Plakoglobin
- β-catenin
- α-catenin
You are studying the assembly of intercellular junctions. You are particularly interested in understanding how the association of different armadillo proteins with various cadherins leads to the differential cytoskeletal associations that are observed in adherens junctions and desmosomes. As part of these studies, you have constructed a series of wild type and chimeric cadherins that can be expressed in epithelial cells and tested for protein interactions using co-immunoprecipitation approaches. The chimeric cadherins are shown in Panel A and comprise either the extracellular or cytoplasmic domains of E-cadherin or the desmosomal cadherin desmoglein.

The results of a yeast two hybrid experiment are shown in Panel B. The plus sign indicates that the proteins interacted and the minus sign indicates that no interaction was observed. Note that no chimeras were used in this two hybrid experiment. Panel C represents the results of a co-immunoprecipitation study in which you determined the association of various catenins with the cadherins shown in Panel A. In this experiment, the cadherin was expressed in an epithelial cell line and then immunoprecipitated under mild detergent conditions to preserve interactions. Western blot analysis was carried out for plakoglobin, β-catenin, or α-catenin.

Questions:

A) (3 points) Based on the results of the yeast two hybrid experiments alone, draw a model of E-cadherin and desmoglein interactions. Diagram the possible associations between these cadherins, plakoglobin, β-catenin and their links to α-catenin based only on the two hybrid results.

B) (4 points) Compare and contrast the results of the yeast two hybrid experiment with the results from the co-immunoprecipitation experiment. Assume that the antibodies used for immunoprecipitations do not interfere with the interactions. Provide an interpretation of the co-immunoprecipitation and western blot results. In addition, identify the implications of these results with respect to the specificity of cadherin interactions with either the actin or intermediate filament cytoskeletal networks.

C) (3 points) Based on these results and your knowledge of intercellular junctions, draw a model of the major cadherins and their binding partners 1) in adherens junctions and 2) in desmosomes.
19) A 50 kb recombinant phage was isolated from a genomic library. In addition to the phage’s long and short arms, the phage carries a 20-kb fragment derived from the Drosophila genome, as diagramed below. Positions of restriction sites are as indicated (B = BamHI; E = EcoRI; H = HindIII; P = PstI).

\[
\begin{array}{ccccccc}
10 & 20 & 30 & 40 & 50 \\
\uparrow & \uparrow & \uparrow & \uparrow & \uparrow \\
H & E & P & E & B & PH & E
\end{array}
\]

A) On the diagram of the agarose gel below, draw the DNA fragments you would expect to see after the specified digests.

\[
\begin{array}{cccccc}
E & H & B & P & E+P \\
50 kb & & & & \\
40 & & & & \\
30 & & & & \\
25 & & & & \\
20 & & & & \\
15 & & & & \\
10 & & & & \\
5 & & & & \\
1 & & & & \\
\end{array}
\]

B) The cloned 5 kb EcoRI fragment was radioactively labeled and used to probe a Southern blot of the gel. On the above diagram, circle each fragment that would hybridize to the probe.

C) Now consider RNA analysis. Briefly compare and contrast Northern Blotting and quantitative real-time(RT)-PCR by discussing one similar application that they can be used for and one advantage of each over the other.

Note: please include this page as part of your answer!
20. A) (4 points) A replication bubble representing a bidirectional DNA replication fork is shown below. The dots within the bubble correspond to the origin of replication, and the arrowheads correspond to 3’ ends. Diagram the leading and lagging strands synthesized at each fork, assuming that each lagging strand is composed of two Okazaki fragments. Indicate the 3’ ends of all DNA molecules with an arrowhead and indicate RNA primers with a wavy line.

B) (2 points) The major replicative polymerase has acquired a mutation that compromises its 3’ to 5’ exonuclease activity but does not affect its polymerization activity. What would be the biological consequence of such a mutation?

C) (2 points) The ends of eukaryotic chromosomes contain simple repeats that are very G-rich on one strand. What is the relevance of these repeats to DNA replication?

D) (2 points) DNA damage not only has the potential to be mutagenic, but also has the potential to stall/arrest DNA replication forks. Briefly describe a mechanism that is used to bypass or tolerate such DNA damage so that replication can continue.

NOTE: Please include this page in your answers!
21. (10 points) Describe the concept of receptor desensitization in the context of G protein-coupled receptors. Distinguish between homologous and heterologous desensitization. Provide details about what is known of two molecular mechanisms responsible for desensitization. How is desensitization reversed?

22. Design and discuss an experiment to test the hypothesis that loss of tubulin subunits at the minus end of kinetochore microtubules is directly responsible for microtubule shortening and chromosome movement during anaphase A of mitosis.

Illustrate as required and include in your answer the following:

A) (2 points) a description of microtubule organization in the metaphase mitotic spindle
B) (2 points) the roles of the centrosome and kinetochore in microtubule assembly and microtubule attachment
C) (2 points) an explanation of tubulin "treadmilling"
D) (2 points) a test of your model for chromosome movement and discussion of possible outcome and
E) (2 points) brief discussion of the source of energy that drives microtubule dynamics and chromosome movement.

23. You have cloned a cDNA from a human liver library that encodes a protein of unknown function. Based on the cDNA sequence, you synthesize a polypeptide containing 10 amino acids and immunize a series of rabbits using this peptide conjugated to an appropriate carrier molecule. When the cDNA is expressed in E. coli, you fortunately find that the rabbit antiserum identifies a single protein band on SDS-PAGE and western blotting corresponding to an apparent molecular weight (Mr) of 80,000. However, when this same cDNA is expressed in a mammalian cell system, the protein exhibits a Mr of 90,000.

A) (9 points) Offer a plausible hypothesis for the discrepancy in Mr between the two proteins obtained in these two different expression systems, and indicate how you might experimentally verify your hypothesis. Be sure to include controls.

B) (1 point): Briefly explain the difference between the molecular weight (Mr) and the molecular mass of a protein. What are the units for each of these?
24. A) The affinity of two interacting macromolecules can be described by the dissociation constant of the intermolecular complex. What is the mathematical formula for the dissociation constant in terms of the concentrations of free ligand, free receptor and complex?

B) What is the mathematical formula for the dissociation constant in terms of the rates at which the complex forms and dissociates?

C) What is the mathematical formula for the dissociation constant in terms of the free energy associated with complex formation?

D) The stability of an enzyme-substrate complex is generally described by a Michaelis constant ($K_M$). How does the Michaelis constant differ from the dissociation constant (in qualitative or mathematical terms)?

hint:

$$ E + S \xrightarrow{k_i} ES \xrightarrow{k_{-1}} P + E $$

$$ K_M = \frac{k_{-1} + k_2}{k_i} $$

E) One of the simplest intermolecular interactions is that of a carboxylic acid group (-CO$_2$-) and a proton (H$^+$). The affinity between these species can also be described as a dissociation constant, but is more typically described by the pKa term of the Henderson-Hasselbalch reaction:

$$ pH = pKa + \log \frac{[A^-]}{[HA]} $$

The gamma carboxyl group of glutamic acid has a pKa of 4.3. If you had a dilute solution of glutamic acid that was buffered at pH 5.0, what fraction of these groups will be unprotonated?

F) As noted above, the affinity of a functional group for a proton is generally described by a pKa value. Amino acids and proteins are instead associated with a pI value. What does pI mean?