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If you have questions please contact David Pallas (4125 RRC) 7-5620.
1) The protein product of Gene X (protein X) is a protease that is proposed to function as a tumor suppressor. The activity of Protein X is diminished in many types of tumor cells including breast cancer. You have obtained a full-length cDNA of Gene X and have chosen to study mutations in Gene X that may lead to tumorigenesis. The first experiment you performed was northern hybridization analysis. You analyzed five breast cancer samples (BC 1, 2, 3, 4, and 5) in parallel with normal breast tissue using the above cDNA as your probe and obtained the results illustrated in A. You next performed western blot analysis using an antibody specific for Protein X and obtained the results shown in B.

A) What is the most likely genetic lesion in Gene X in each of the above breast cancer samples? (0.5 points each=2.5 points total)

B) Provide a BRIEF experimental design to test your hypothesis in each case. (0.5 points each=2.5 points total)

C) Please design TWO additional experiments (2.5 points each) to test the hypothesis that Protein X is indeed a tumor suppressor. Assume you have the necessary (reasonable) reagents. Describe predicted results and discuss potential drawbacks.
2) An important technique for studying microtubule-associated motors is to attach the motor proteins by their tails to a glass cover-slip and then to allow microtubules to settle on the motor covered surface. In the light microscope the microtubules can be seen to move over the surface in an ATP dependent manner as the motor domain propels the microtubules.

A) Since the motor proteins attach to the cover-slip surface in random orientations, how do the motors generate coordinated directed movement of each microtubule rather than engaging in a hopeless tug of war with no movement of microtubules? Illustrate the basis for your explanation. (5 points)

B) Relative to microtubule polarity, which direction will microtubules move when placed on a dynein coated surface? Include in your answer a description of how the direction of microtubule movement would be determined in this in vitro experiment. Illustrate as required. (5 points)

3) You have been doing a screen to identify DNA sequences that lead to decreased expression of the *EEK1* gene in cultured mouse fibroblasts. You are transforming in plasmids from a mouse genomic library trying to identify those genes that lead to a decrease in the level of *EEK1* mRNA. The library plasmid contains no promoter so the experiment relies on expression from endogenous promoters contained within the genomic sequences in the plasmid. In your screen you identify a single plasmid numerous times. Subsequent experiments convince you that mouse cells transformed with this plasmid definitely show a decrease in the steady-state level of the *EEK1* mRNA. You eagerly sequence the plasmid and you are surprised to find that the genomic region you have cloned does not contain any open reading frames. Further analysis of the sequence does reveal a putative TATA box that is evolutionarily conserved.

A) Describe a hypothesis that explains your results (4 points).

B) Briefly describe TWO experimental approaches that you could use to begin to test your hypothesis (6 points).

4) You are studying a recently discovered restriction endonuclease called Emo RI, which catalyzes the hydrolysis of the double stranded palindromic sequence: 5'-GACGTC-3'. You have purified the enzyme and have determined (in a Bio-Rad protein assay) that the concentration of your stock is 2 micrograms/mL. The predicted molecular mass of the enzyme based on amino acid sequence is 40 kD.

A) What is the molar concentration of enzyme in your stock? Hint: the concentration of any molecule in solution (chemical or macromolecule) can be expressed in molar terms. (2 points)

B) Describe an assay for this enzyme activity. Assume that you have infinite quantities of a 1000 bp DNA fragment containing one internal 5'-GACGTC-3' sequence. (2 points)

C) Suppose you incubate 1 microliter of your enzyme stock with 1 microgram of DNA (1000 bp fragment) in 50 microliters of buffer. The molecular mass of double stranded DNA is 650 daltons per base pair. What are the molar concentrations of enzyme and substrate at the start of the reaction? (2 points)

D) How would you use your assay to determine the steady-state kinetic parameters (Km, Vmax) of Emo RI in reactions with the 1000 bp DNA fragment? (2 points)

E) Restriction endonucleases generally have good Km values (~50 nM) but poor kcat values (~1/sec). Why? (2 points)
5)  

A) You are studying a new plasma membrane protein. Analysis of the protein purified from plasma membrane preparations shows that your protein is 220 kD in size when it is on the plasma membrane surface. However, the predicted molecular size from the primary sequence is 180 kD. Patients with a mutation in the gene encoding this protein show only the lower molecular weight form, which appears to be localized only in the Golgi apparatus. How can the discrepancy in molecular sizes be explained? (3 points) Briefly describe one experiment you would do to test your hypothesis. (1 point)

B) When you are further along in your studies, you find a drug that binds a known site on your novel protein (now known to be a receptor) and alters its function in a manner that may have potential for medical therapeutics. You decide to design a drug with higher affinity binding. When you make a point mutation in the receptor binding site from a leucine to glutamate and measure the kinetics of binding of the drug, you notice that the off rate of the drug from the receptor is faster and the on rate is unchanged.

1. What has happened to the binding affinity? to the Km? Briefly explain your answers. (1 point each)
2. Is the interaction surface between the drug and the receptor at the receptor mutation site most likely electrostatic or hydrophobic? (1 point) Why? (1 point)
3. Does the interaction between the receptor at the mutated point and its complementary surface on the drug occur early or late in the reaction coordinate? (1 point)
4. In one sentence, state how would you design a higher affinity drug for the native receptor by altering the drug? (1 point)

6)  

A) What is the centrosome, and what role does the centrosome play in the cell? (2.5 points)
B) Which stage of the cell cycle do centrosomes duplicate? (2.5 points)
C) How was this determined? (i.e. describe an experiment designed to determine the stage of duplication of the centrosome.) (2.5 points)
D) What is meant by semi-conservative centriole duplication during centrosome duplication? Illustrate as required. (2.5 points)

7) Ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) is a membrane-bound enzyme with its two active sites in the extracellular space and short cytoplasmic tails that may be involved in signal transduction. This enzyme hydrolyzes extracellular NTPs to the corresponding NDP and extracellular NDPs to the corresponding NMP. The enzyme does not have NMPase or non-specific phosphatase activity. Inhibition of the ecto-nucleotidase activities of this enzyme inhibits the growth of cells expressing it. You have a wild-type cell line that expresses this enzyme and a knock-out cell line lacking the enzyme, which grows more slowly than the wild-type cells. Describe TWO approaches to test whether the ecto-NTPase activity, the ecto-NDPase activity, or both, mediate the growth regulatory function by hydrolyzing extracellular nucleotides. Be sure to include proper controls. (5 points each approach)
8) Chromatin remodeling is the basis of all aspects of gene regulation.
   A) List FOUR different mechanisms that lead to chromatin remodeling and indicate whether these changes result in gene activation or silencing. (0.5 points each)
   B) Describe TWO of these mechanisms (2 points each) in more detail, including aspects of regulation and mechanism.
   C) Discuss TWO methods for determining that the relationship between nucleosomes and DNA has been altered. (2 points each)

9) A polymer is something made of multiple (‘poly’) repeating units (‘mers’). In cells there are both covalent polymers and non-covalent polymers. The repeating units can be identical (homopolymers) or different (heteropolymers).
   A) List FOUR DIFFERENT types of cellular polymers whose repeating units are complex (for example, not just -CH2-). Include at least ONE covalent polymer and ONE non-covalent polymer. In each case, state what the monomeric building block is. (0.5 points each)
   B) Briefly describe THREE advantages of SPECIFIC polymers over monomers that make them so useful to living organisms and give ONE clear cellular example for each characteristic. (1 point for each advantage/example pair)
   C) Why does a mammalian cell need covalent and non-covalent polymers? (Give TWO reasons why the cell cannot use one type for all purposes?) (1.5 point each)
   D) Give FOUR examples of cellular processes that employ polymers. (0.5 points each)

10) You have purified a protein called YDUIT from mammalian fibroblast cells. No full-length cDNA clone for this protein is available commercially.
    A) Describe the detailed steps of how you would obtain a full-length cDNA clone that encodes this protein. (7 points)
    B) Describe THREE reasons why it would be useful to obtain and sequence the cDNA even though you had access to the purified protein. (1 point each)

11) You have preliminary data that the transcription factors MARS1 and VENUS2 regulate expression of the gene cosmic. Increased MARS1 results in increased cosmic, but increased VENUS2 results in decreased cosmic. However, you do not know whether they act directly or indirectly. You have full-length cDNA clones of the MARS1 and VENUS2 genes, but no good antibody to either protein exists.
    A) Outline a set of experiments that will enable you to generate the necessary reagents and then use those reagents to determine whether MARS1 and/or VENUS2 directly regulate cosmic. (4 points)
    B) Propose mechanistic models to explain your data if it shows that

       a. MARS1 directly regulates cosmic, but VENUS2 does not. (1.5 points)
       b. VENUS2 directly regulates cosmic, but MARS1 does not. (1.5 points)
       c. Both MARS1 and VENUS2 directly regulate cosmic. (1.5 points)
       d. Neither MARS1 nor VENUS2 directly regulate cosmic. (1.5 points)
12) Age-related declines in stem cell proliferation and function are observed in numerous tissues. You hypothesize that such impairments in stem cell function are due to changes in secreted factors in the circulation. You set up an experimental system in which regenerating tissues in old mice are exposed to the circulatory system of young animals, and vice versa. As shown below, you establish parabiotic pairings between young and old mice, with parabiotic pairings between two young mice or two old mice as controls. In parabiosis, animals develop vascular anastomoses and thus a single, shared circulatory system.

After five weeks of parabiotic pairing, BrdU is injected into the mice several days before sacrifice and collecting the livers. The number of BrdU positive cells is analyzed in liver sections and plotted in the graphs below. Assume that you have done preliminary experiments and have shown that circulating cells are not responsible for the data below.

A) Suppose you obtain the results seen in Graph A. What conclusions regarding aging can be drawn about secreted systemic factors? (3 points)

B) Suppose instead that you obtain the results seen in Graph B. What conclusions regarding aging could you draw about secreted systemic factors? (3 points)

C) You separate the mice used to obtain the results in Graph B and restore normal circulation to these animals. After two weeks, you test again and obtain the same data for each of the individual mice that you observed in graph B when the mice were still joined. Propose a hypothesis to explain this observation. (3 points) What commercial implications might this have? (1 point)
Day Two (The End is Near!) June 2\textsuperscript{nd}, 2005

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13) The following N-terminal, amino acid sequences were obtained for two different, soluble proteins purified from a total liver homogenate from rat liver:

X. NH$_3$-Met-Ala-Val-Arg-Leu-Gly-Ala-Lys-Leu-Leu-Val-Lys-Trp-Ala-Val-Arg-Val-Leu-
Y. NH$_3$-Met-Ala-Val-Ile-Leu-Gly-Ala-Ala-Leu-Leu-Val-Phe-Trp-Ala-Val-Ala-Val-Leu-

A) Assuming that each of these N-terminal amino acid sequences targets the respective protein to a particular organelle in the cell, predict to which organelle each protein is targeted. Explain your answer. Include in your answer any predicted differences in the mechanisms of import into these organelles. (4 points)

B) Design an experiment to test your prediction that the given sequences are both necessary and sufficient to target the proteins to the predicted organelles. (3 points)

C) Is there sufficient information contained in the two sequences to predict the final steady-state organelle location? Explain your answer. (3 points)

14) Shown below is the region of DNA containing a Drosophila gene, divided into 10 segments designated A-J.

A) What segment(s) of the DNA will be represented in the initial RNA transcript? (Note: no partial credit will be given.) (2 points)

B) What segment(s) of the DNA will be found in the completely processed transcript? (2 points)

C) What segment(s) of the processed transcript will have additional information added that is not specified by the DNA? State in a single phrase the modification to each segment(s). (4 points)

D) Draw in your answer what you would expect to see in an R-loop experiment if the transcribed strand of DNA is hybridized to the completely processed RNA transcript. Label the DNA strand in your picture. (2 points)
15) Your lab has used up all the MreII restriction enzyme. Due to an extreme shortage of funds (curse the NIH), you have been charged with the responsibility of purifying more of this enzyme. You have a strain of bacteria that highly expresses this protein but it is not yet cloned. Unfortunately, the protein is not tagged and there is no money to perform any molecular biology to create a tag or to clone the enzyme. Thus, you must use (gasp) classical biochemistry to purify the protein. Following are known properties of MreII:
MW 150 kDa
PI 8.7

A) Please describe how you will accomplish this process (the strategy rather than the experimental details). Start with how you will lyse the bacterial cells and then provide a TWO-step purification. Please provide rationale for each of your proposed purification steps. (6 points)
B) What measurements will you need to make at each step of your purification to assure yourself that you have enriched for MreII? (2 points)
C) Finally, what is your assay for MreII and what are the critical properties of this activity assay? (2 points)

16) You have been studying the function of an interesting protein. You have generated a model where your protein of interest functions by heterodimerizing with another protein.
A) Describe an experiment that you could carry out to determine if these two proteins indeed interact (5 points). Assume that all the necessary reagents are available. Be sure to include appropriate controls.
B) Assume you find a direct interaction between the two proteins. Briefly describe ONE method that you could use to calculate the binding affinity of the two proteins (3 points).
C) Suggest ONE experimental approach that you could use to begin to test whether the interaction that you have identified is critical for the in vivo function of your protein (2 points).

17) The Sodium-Potassium ATPase (Na⁺-K⁺ Pump) is responsible for producing the membrane potential across most animal cells.
A) What does the Na⁺-K⁺ Pump do and how does this produce a membrane potential? (4 points)
B) What happens to the membrane potential immediately after the pump is suddenly inactivated? Explain your answer. (2 points)
C) What happens to the membrane potential after one minute of inactivation? Explain your answer. (2 points)
D) Would the cell swell, shrink or not change volume 30 minutes after the pump is inactivated. Explain your answer. (2 points)

18) Discuss how the insulin signaling pathway contributes to aging. Include in your discussion a description of daf-2 and daf-16. Finally, discuss some of the downstream genes affected by activation and/or inactivation of this pathway. (10 points)
19) A) A vast number of cellular proteins are degraded by the ubiquitin-proteasome system. Since nonspecific degradation of proteins by the proteasome could be deleterious to cells, a variety of mechanisms is used to ensure specificity. Briefly explain TWO of these mechanisms (4 points).

B) Ornithine decarboxylase (ODC) is an enzyme that catalyzes the rate-limiting step in polyamine biosynthesis. In the presence of polyamines ODC has a short half-life. Using pharmacological approaches (drugs), design experiments to define which of the following cellular pathways of proteolysis is involved in degrading ODC: proteasome, lysosome, calpains, caspases. Be sure to include controls. (3 points) What are the limitations of this approach? (1 point)

C) Assume that you find ODC is degraded by the proteasome. Briefly describe how you would determine whether ubiquitination is required? (2 points)

20) In mammalian cells, 4E-Binding Protein (4EBP) binds and inhibits eukaryotic initiation factor 4E (eIF4E), thus affecting cellular control of translation. Activation of mTOR kinase blocks the ability of 4EBP to bind and inhibit eIF4E function. Assume for parts A-D below that you have the necessary reagents.

A) Design an experiment to test whether mTOR can phosphorylate 4EBP directly. (3 points)

B) Addition of the drug, rapamycin, to cells greatly reduces 4EBP phosphorylation. Assume that you found that mTOR directly phosphorylates 4EBP. You hypothesize that rapamycin inhibits mTOR kinase activity. Design an experiment to test this hypothesis. Unfortunately, you know that rapamycin does not inhibit bacterially-expressed recombinant mTOR so you cannot use recombinant mTOR for your experiment. (3 points)

C) Assume that you found that rapamycin can inhibit mTOR kinase activity in mammalian cells. Yet, you already know rapamycin does not inhibit recombinant mTOR from bacterial cells. Propose an explanation for this difference that does not involve a difference in covalent modification of mTOR between the two sources. (2 points)

D) Design an experiment to test whether rapamycin can enhance 4EBP-mediated suppression of eIF4E in living cancer cells. (2 points)

21) A) What are FOUR things that control protein folding? (4 points total)

B) Why is it so difficult to design computer algorithms to determine how a protein will fold in vivo? (3 points)

C) If a protein is deliberately denatured in vitro by suspension in a chaotropic solution (8M urea containing β-mercaptoethanol, for instance) will it be able to re-fold properly and, if so, how will this occur? If not, why not? (3 points)
22) Flossie, your champion milk-producing cow, is the main money producing cow in your herd. You wish to clone her using somatic nuclear transfer. Given your understanding of somatic nuclear transfer, discuss the following:

A) What is the purpose of the diploid nucleus derived from Flossie? (2 points)
B) Why is the recipient egg enucleated before the procedure? (2 points)
C) What is left in the recipient egg after enucleation and why is this important for the developing animal? (2 points)
D) What is the purpose of the electric shock? (2 points)
E) Your cloning experiment succeeds beyond your wildest dreams and 3 female calves, Missie, Mossie and Minnie, are born from surrogate mothers. However, you are surprised that the 3 calves all look different from each other and from Flossie. What is going on here? (2 points)

23) Many developing systems employ G-protein-linked Receptors or Receptor Tyrosine Kinases in signal transduction.

A) Name ONE specific example of each of these two types of receptor (1 point each).
B) Discuss FOUR general features (1.5 points each) that contribute to their highly effective role as signal transducers.
C) What is the difference between a Receptor Tyrosine Kinase and a Tyrosine Kinase-Associated Receptor? (1 point) Give an example of a Tyrosine Kinase-Associated Receptor. (1 point)

24) Below is an abstract from a recent paper. Certain findings are lettered A-E and underlined. Choose 4 of the 5 findings and for each describe in 1-2 sentences the key experiments you would have carried out to reach these conclusions. Also for each finding, briefly list key controls and their purpose. (2.5 points each)

Granzyme A induces caspase-independent mitochondrial damage, a required first step for apoptosis. Martinvalet D, Zhu P, Lieberman J.
The CBR Institute for Biomedical Research and Department of Pediatrics, Harvard Medical School, Boston, MA 02115, USA.

Granzyme A (GzmA) triggers cell death with apoptotic features by targeting the endoplasmic reticulum-associated SET complex, which contains the GzmA-activated DNase NM23-H1, its inhibitor SET, and Ape1. The SET complex was postulated to translocate to the nucleus in response to oxidative stress and participate in its repair. Because mitochondrial damage is important in apoptosis, we investigated whether GzmA damages mitochondria. A) GzmA induces a rapid increase in reactive oxygen species and mitochondrial transmembrane potential loss. B) but does not cleave Bid or cause apoptogenic factor release. C) The mitochondrial effect is direct, does not require cytosol, and D) is insensitive to bcl-2 and caspase inhibition. E) SET complex nuclear translocation, which occurs within minutes of peroxide or GzmA treatment, is dependent on superoxide generation since superoxide scavengers block it. Superoxide scavengers also block apoptosis by CTLs expressing GzmA and/or GzmB. Therefore, mitochondrial damage is an essential first step in killer cell granule-mediated pathways of apoptosis.