Problem Set 4
7.06
Spring 2007

Question 1.
Answer the following questions about actin and microfilaments.

(a) What happens (net growth, net shrinkage, or no net change) to filament length when microfilaments are incubated at each of the following concentrations of monomer?

…a monomer concentration equal to the Cc for the (–) end?

…a monomer concentration equal to the Cc for the (+) end?

…a monomer concentration equal to the Cc for the filament?

(b) What happens (net growth, net shrinkage, or no net change) at each end when microfilaments are incubated at each of the following concentrations of monomer?

	PLUS	MINUS
…a monomer concentration equal to the Cc for the (–) end?		
…a monomer concentration equal to the Cc for the (+) end?		
…a monomer concentration equal to the Cc for the filament?		

(c) Cytochalasin D and latrunculin both lead to the destabilization of microfilaments. However cytochalasin D binds to F-actin and latrunculin binds to G-actin. Explain how this difference in mechanism can still lead to the same effect.

(d) How can you demonstrate experimentally that ATP hydrolysis by actin is not necessary for a subunit to add on to a microfilament?

(e) Why is it, if the concentration of ATP is much higher in the cell than ADP and the ATP-bound form of actin is more likely to polymerize, that not all the actin in the cell is polymerized?

Question 2.

Embryonic epithelial cells grown in rich culture medium appear round and symmetrical. Moving the cells to nutrient-poor medium will cause the cells to differentiate and become polarized.

(a) When you move embryonic epithelial cells to nutrient-poor medium in the presence of cytochalasin D, you find that the cells remain round and symmetrical. What can you conclude from these results?

(b) You wish to visualize the organization of actin in polarized epithelial cells. How could you visualize actin?

(c) Microvilli are cellular projections located on the apical surface of some epithelial cells (such as intestinal epithelial cells) that increase surface area. They contain a core of actin filaments arranged in bundles. You suspect that these bundles are maintained by actin-bundling proteins. Actin-bundling proteins are one of the many kinds of actin-binding proteins we learned about in class. In general, how might you isolate and identify actin-binding proteins?

(d) Once you have identified actin-binding proteins by way of part **(c)**, what experiments might you use to see whether it is possible that these proteins are specifically actin-bundling proteins?

Question 3.
You are a UROP student working in a cancer research lab. You were told that, except for blood cells, all normal cells in your body are anchorage-dependent, meaning that they

have to grow in or on some sort of extracellular matrix. If cells are deprived of extracellular matrix, they will not proliferate in the presence of growth factors, and these cells will die by apoptosis.

(a) What can you conclude are two functions of ECM proteins, apart from being the "glue" that holds the cells of your body together?

(b) What is the danger in the ability of some cancerous cells to grow in an anchorage-independent manner?

In your lab, there are two mammary epithelial cell lines available; the two cell lines are called S1 and T4-2. S1 is a non-malignant (non-cancerous) cell line, whereas T4-2 is a cancerous cell line. You grow both cell lines in regular tissue culture dishes (this is considered 2-D culture, and the cells stick to the plastic dish) and both cell lines look the same. By morphology, you cannot tell which cell line is which.

However, another lab has established a 3-D culture system, where you mix your cells with different extracellular membrane (ECM) proteins, and let the cells grow within the environment of these ECM proteins. You grow S1 cells in ECM proteins from basement membrane and find that the cells reorganize into spheroids with a hollow lumen. (The basement membrane is the basal lamina we learned about before and the adjacent layer of collagen.) These spheroids look like the structures that mammary epithelial cells adopt in the whole organism, where the cells organize into hollow tubules and secrete milk into the inside of the tubules.

(c) How could you test whether the cells in these spheroids are polarized and aligned properly?

Your result from part **(c)** tells you that these cells are indeed polarized and properly oriented when grown with ECM proteins from the basement membrane. You are interested in examining the effects of different ECM proteins on these cells, and you know that basement membrane ECM and interstitial ECM contain different proteins. (Interstitial ECM is the kind of ECM that holds cells of an organ all together.) When you grow the S1 cells in interstitial ECM proteins, you do not see the formation of spheroid structures or properly polarized cells. Rather, you see significant proliferation of S1 cells, and those cells grow in non-descript clumps of cells.

(d) What is the significance of the fact that you see significant proliferation of S1 cells? Do you think that the S1 cells were proliferating in the experiment using basal membrane ECM proteins? Explain your answers.

(e) How would you explain the difference in results using interstitial ECM proteins and basement membrane ECM proteins? State your hypothesis.

Now you grow T4-2 cells in basement membrane ECM and get the following results. Whereas S1 cells form spheroids in this assay, T4-2 cells grow into a clump of cells without normal structure.

S1 cells   T4-2 cells

(f) State a hypothesis for the difference in results between S1 and T4-2 cells grown in basement membrane ECM proteins.

(g) You decide to treat the two different cell populations with two specific antibodies you have against different integrins, namely beta1 and alpha6/beta4. The two antibodies you have are "inhibitory antibodies" because, when they bind to their substrates, they inhibit the function of their substrates. You are trying to decide whether you need to permeabilize cells before adding these antibodies to the cells. What do you decide and why?

When you treat the different cell types grown in the presence of basement membrane ECM proteins with or without inhibitory antibodies against specific integrins, you obtain the following results:

T4-2 cells No antibody	T4-2 cells anti-beta1 integrin	S1 cells No antibody	S1 cells anti-alpha6/beta4

(h) What do you conclude from these results?

Question 4 .

(a) You are a grad student studying genetic alterations that lead to tumorigenesis. While plating primary cell cultures from a healthy person and a tumor from a cancer patient, you mix up the samples. A colleague suggests that you use the differences in growth properties between the two samples to distinguish them. Describe how the growth properties of cancer cells differ from those of healthy primary cells.

(b) In addition to differences in growth properties, cancer cells also have morphological differences, such as a disorganized cytoskeleton. The Rho family of small GTPases is involved in cytoskeleton regulation, and the pathways that Rho proteins are involved in are implicated in cancer development. Their regulation of the cytoskeleton is thought to be important in cell migration during cell division and invasion of other tissues. In an experiment performed a decade ago, it was found that transfecting NIH 3T3 cells with a construct that expressed a specific mutant form of RhoA could transform these cells. Based on this experiment, would you predict that RhoA is a proto-oncogene or a tumor suppressor gene? Would you predict that the RhoA mutation is a gain-of-function mutation or a loss-of-function mutation? Explain your answers.

(c) In class, we learned about the experiment by the Weinberg lab, in which an oncogenic version of Ras was discovered in cells from a human bladder carcinoma. This was done by transfecting human carcinoma DNA into NIH 3T3 cells (which is a cell line that can grow indefinitely) and looking for the formation of foci (clumps of cells that grow on top of each other).

When oncogenic Ras is transfected into Rat Embryonic Fibroblast (REF) cells (a wild-type primary cell culture), it could only partially transform the cells. However, if a hyper-active mutant version of the myc gene is introduced into the REF cells along with the oncogenic version of Ras, the cells are fully transformed. Why did the REF cells require another oncogene to become transformed, while NIH 3T3 cells did not?

(d) Human papillomaviruses (HPVs) are a family of DNA viruses that cause genital warts. HPVs have been predominantly associated with cervical cancer. One of the proteins encoded by the HPV virus is E5. E5 is a short transmembrane protein that forms a dimer or trimer. E5 can form a stable complex with endogenous host PDGF receptor. Explain how this could lead to cell transformation.

(e) HPV also encodes proteins E6 and E7. It has been shown that adding E6 and E7 to normal cells is sufficient to transform them and induce uncontrolled mitosis. Initially, it was not clear how E6 and E7 accomplished this, but recently it has been demonstrated that E6 binds and inhibits p53, while E7 is known to bind and inhibit Rb. Why do these findings explain how HPV causes warts (which are benign tumors), and

how HPV infection increases one's risk of developing cancer?

Question 5.

You are studying some patients with Li-Fraumeni disease, in whose families many members have various forms of cancer. Your supervisor tells you that this disease is the consequence of an inherited mutation in the p53 gene.

(a) If you could analyze cells from a male patient afflicted with Li-Fraumeni disease, how many wild-type and how many mutant copies of p53 would you expect to find in each cell if you analyzed:

…10 non-cancerous somatic cells?

…10 tumor cells?

…10 sperm cells?

(b) You determine that loss of heterozygosity has occurred in the patient's tumor cells. List all possible cellular events that could have triggered this process to occur.

(c) Why is it that cells with a faulty spindle assembly checkpoint undergo loss of heterozygosity more frequently than cells with an intact checkpoint?

(d) You sequence both copies of p53 in the patient's tumor cells, and find that there are two copies of the gene present and they both consist of the exact same DNA sequence. This result should restrict the number of possibilities you listed in part **(b).** State which of your theories of possible cellular events from part **(b)** are still feasible.

(e) How would looking at the DNA sequence of genes that are farther up and down on chromosome (but have nothing to do with cancer development) help you to distinguish between the remaining possibilities listed in part **(d)?**

Question 6.
You are working in a cancer research lab studying tumorigenesis in mice.

(a) There are lots of genes that your lab might study! List all of the genes in the following pathways that we have learned about throughout the semester that are oncogenes, and all of the genes that are tumor suppressor genes.
i) The Wingless pathway
ii) The RTK/Ras pathway

(b) One of the assays your lab uses is the soft agar colony forming assay. What is the principle behind this assay?

(c) One of the genes studied in your lab is E2F, which is a transcripton factor that controls the G1 to S transition in mammals.
i) How is E2F normally regulated in the cell?

ii) How can E2F be inappropriately activated to lead to cancer?

iii) How could you show experimentally that an excess of free E2F can lead to tumorigenesis? (Make sure to include controls in your experiment.)

(d) Another of the genes studied in your lab is Ras. Which of the following changes to wild-type Ras would make it oncogenic?
-- if Ras cannot interact with Raf
-- if Ras cannot hydrolyze GTP
-- if Ras cannot interact with Sos
-- if the farnesyl lipid anchor cannot be added to Ras

(e) You have a mouse strain that is predisposed to developing skin cancer. Based on the literature in the cancer field, you come up with a list of 10 potential candidate genes that might be mutated in these mice. You are convinced that the mice of this strain must harbor one of these mutations. How would you determine which candidate gene from the list was actually mutated in this mouse?

(f) When you do the experiment listed above to determine which candidate gene is responsible for the predisposition to developing skin cancer, do you need to take tissue samples from the tumor itself, or can you take tissue from anywhere in the mouse?

(g) You also have a large colony of wild-type mice that you maintain in the lab. Once of these mice develops a disease that looks very similar to chronic myelogenous leukemia (CML). Knowing that a mutation in the *c-abl* gene often leads to CML in humans, you decide to test your tumorigenic mouse for mutations in *c-abl*. You design primers upstream and downstream of the *c-abl* open reading frame and try to perform PCR to amplify the *c-abl* gene from cancerous cells that you have harvested from your tumorigenic mouse. When you do PCR from a wild-type mouse on *c-abl*, the PCR reaction works just fine. You cannot get a *c-abl* PCR product from your tumorigenic mouse, however, no matter how much tweaking of reaction conditions you do. Explain why you can't get a PCR product for *c-abl* in the tumorigenic mouse.

(h) Knowing what you know about the *c-abl* oncogene, how could you use PCR to test for the mutation in your tumorigenic mouse?

Question 7.
You are interested in studying the biology of stem cells.

(a) Explain the difference between embryonic stem cells and adult stem cells. Where would you find each kind of stem cell? How many different cell types can each category of stem cells generate, relative to one another?

(b) A patient has a rare recessive genetic disease due to a loss of function in a specific gene. This disease causes a severe immunodeficiency in the patient. The patient receives a bone marrow transplant, which is successful in alleviating the symptoms of the immunodeficiency disease, and the patient is now in good health. Why did the bone marrow transplant relieve the patient's symptoms?

(c) Your patient marries a man who is a carrier for the same genetic disease. How do you counsel your patient on the probability of their children inheriting the disease, given that she no longer shows any symptoms of the disease?

(d) One can generate an organismal clone of a sheep by somatic cell nuclear transfer. To do this procedure, you take the nucleus from a cell from "Sheep A" and insert it into the cytoplasm of an enucleated egg taken from "Sheep B." You then implant the newly-nucleated egg into pseudopregnant "Sheep C." You do this procedure, and you name your resulting newborn clone "Sheep D." The cell you took from Sheep A in your experiment was a cell from the immune system that makes antibodies. When Sheep D is born, it is severely immuno-compromised. Sheep D is capable of producing antibodies, but only one kind of antibody against one specific antigen. Normally, an organism can produce an enormously large number of different kinds of antibodies, each one of which can recognize a different antigen. What does this result suggest?

Question 8.

You are working in a lab that is trying to successfully perform stem cell therapy on mice with Parkinson's Disease by inducing stem cells to become nerve cells in vitro, and then delivering these nerve cells to the diseased mouse's brain. The mouse you are trying to treat has a form of Parkinson's Disease that is autosomal recessive and is caused by loss-of-function in the PARK2 gene.

(a) What is a potentially life threatening problem with treating the diseased mouse with stem cells taken from another mouse who is not related at all to the diseased mouse?
(b) What are two potential major problems specific to trying to use adult stem cells for your stem cell therapy experiment?

(c) You decide that you want to work with ES cells that are genetically identical to those of the diseased mouse, due to the problems listed in parts (a) and (b). Why can't you directly isolate such ES cells from the diseased mouse?

(d) You decide to use Somatic Cell Nuclear Transfer in order to derive ES cells that are genetically identical to the diseased mouse. List the steps of the procedure you would need to do to accomplish this.

(e) Before you induce the ES cells you derived in part **(d)** to become nerve cells, what must you do to the ES cells to make the stem cell treatment actually beneficial to the diseased mouse? (HINT: Re-read the introduction to this question.)