Sample Exam 2

Feel free to use sketches when this is helpful to explain your answers.

1) [8 points] a) Describe the experimental observations that together indicate that calcium ions mediate a regulatory step after fertilization of animal eggs.

b) What events do Ca^{++} ions cause in the fertilized egg?

2) [10 points] Suppose the following proteins are required for a particular signal transduction pathway. a) Indicate the sequence in which they probably act after perception of a signal.

i) Ca^{++} channel
ii) transcription factor
iii) receptor
iv) G-protein
v) phospholipase C

b) At what location in the cell would you expect to find each of these proteins?

3) [20 points] a) On the following diagrams of Xenopus embryos at two different stages, indicate i) the blastocoel, ii) the location of cells that will give rise to the stomach, iii) the location of cells that will give rise to the heart.

b) On the following diagrams of human embryos at two different stages, indicate i) the blastocoel, ii) the location of cells that will give rise to the stomach, iii) the location of cells that will give rise to the placenta.

4) [23 points] An experimentalist isolates stem cells from adult mouse skin and wants to grow them in culture. However, she finds that as soon as she isolates them they differentiate spontaneously into skin cells.
a) Can you suggest a strategy to keep the stem cells in an undifferentiated state and therefore amenable to experimentation?

b) If she can prevent them from differentiating spontaneously, how might she then treat them to induce them to differentiate into a mesodermal cell type?

c) Suppose she wants to study how gene expression changes during differentiation of these cells into skin. Suppose she has a microarray with spots for 20,000 mouse gene sequences.

i) How many of these genes do you expect will change in expression during the transition from stem cell to skin?

a) 10
b) 50
c) 500
d) 20,0000

ii) How many hybridization experiments would you have to perform to obtain the result in part i if you used Northern hybridization? Explain your answer.

iii) How many hybridization experiments would you have to perform to obtain the result in part i if you used a microarray? Explain your answer.

d) Suppose that you have available a gene whose promoter drives expression specifically in the skin stem cells. Diagram a DNA construct that you could use to express GFP (Green Fluorescent Protein) in skin stem cells.

e) Outline the steps you would perform to make transgenic mice carrying this construct.

f) Outline experimental steps by which you could use these transgenic animals to determine which genes are expressed in skin stem cells in a mouse.

5) [15 points] The extracellular small protein Wnt11 is a candidate signal required for specification of dorsal mesoderm early in development of the amphibian *Xenopus laevis*. Other closely related molecules of the Wnt family act by causing nuclear localization of a protein called β-catenin, which then regulates gene expression. (See canonical pathway sketch.)

Suppose you wanted to test whether Wnt11 is indeed the candidate signal that induces dorsal mesoderm formation. Describe experiments you could use to test i) whether Wnt11 is present in the right time and place to be the signal; ii) whether Wnt11 activity is sufficient to induce a dorsal axis in intact embryos; and iii) whether Wnt11 is necessary for dorsal mesoderm formation in intact embryos. In each case, describe the methods you could use and what results you might expect to obtain.

i) Right time and place:

ii) Sufficiency:

iii) Necessity:

6) [24 points] a) Diagram the general structure of human skin. (You may ignore the hair.) Indicate in your diagram the skin stem cells, which cells are most differentiated, and which cells are alive.

b) In the inherited predisposition to skin cancer Xeroderma Pigmentosum (XP), a mutation in any of several genes can cause predisposition to cancer. In any given family, this condition is inherited in a similar manner as Hereditary non-polyposis colorectal cancer (HNPCC).
What biochemical or signaling event(s) do wild-type XP proteins carry out in normal cells?

c) Why do patients with XP preferentially develop skin cancer instead of other cancers such as colon cancer?

d) What is likely to be the first event leading to skin cancer in someone with the inherited predisposition to XP?

e) Sketch a patch of skin in someone with a predisoposition to XP in which a cancer is developing. i) Indicate which cells are different from the corresponding cells in part a. ii) How are they different?