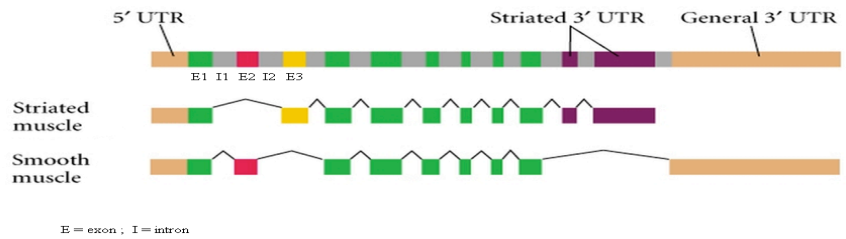
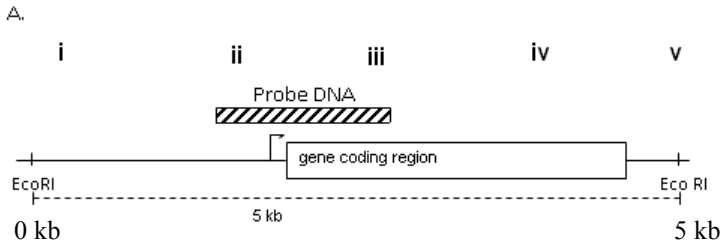
MCDB 4650 Problem Set 1

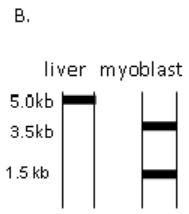


1. (1) The gene diagrammed is the tropomyosin gene, expressed in a wide variety of cell types. Give one possible reason (and explain briefly) why the expression of these two proteins might differ as a result of the alternative splicing shown in the diagram?

2. (1) Which of the following statements is/are true about cell-cell signaling in development, when given a certain concentration of ligand?
   a. The extracellular matrix cannot induce the activation of an intracellular signaling pathway
   b. If the DNA, mRNA and protein products of two cells are identical, they can still respond differently to an extracellular ligand binding a receptor on their surface.
   c. All growth factors work through a single cell signaling pathway
   d. Multiple cell signaling pathways work through the phosphorylation of an intracellular receptor upon binding of that receptor with its ligand.
   e. The binding of a ligand to its receptor cannot result in the inhibition of other proteins associated with that receptor.

You have cloned a gene that encodes a muscle-specific enzyme and determined the transcription start site as indicated in A, and you wish to determine whether there is a methylated CG sequence in the promoter region that becomes demethylated in myoblasts (muscle precursor cells) before the gene is expressed. You extract DNA from myoblasts and, as a control, from liver, and digest both DNAs with EcoRI and HpaI, which cuts CCGG sequences only if the second C is <u>not</u> methylated. You then do a Southern blot with each sample and the labeled probe shown, with the results shown in B.

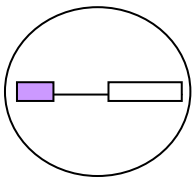
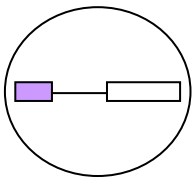




3. (1) Is there a methylated CCGG sequence near the gene in liver that has become demethylated in myoblasts? Indicate by choosing the site:
   a. i, b. ii, c. iii, d. iv, e. v, f. None

4. (1) Are there any other unmethylated CCGG sequences between the EcoRI sites in either DNA? If so, choose their position; if not, choose none.
   a. i, b. ii, c. iii, d. iv, e. v, f. None

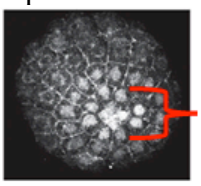
5. (2) Below are the nuclei of two cells, with the same gene shown in each nucleus (shaded box is the regulatory region; open box is an exon). In cell X, this gene is transcribed. In cell Y, this gene is not transcribed. Briefly explain two different ways in which this could occur.

Cell X   Cell Y

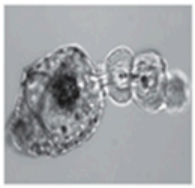
6. (2) You are investigating how cells in a frog embryo differentiate into epithelial (skin) cells or neuronal cells. You do two experiments.
A. Fluorescently label a progenitor cell in the early embryo. Observe the larva 2 days later to see which kinds of cells contain the label (assume you can distinguish between cell types with an antibody).
B. Isolate a progenitor cell in a culture dish with only the correct nutrients to sustain it. Two days later, use molecular techniques to determine what kinds of cells (neuronal, epithelial, or both) are present in the dish.

When you analyze the results, you find that in experiment A, all labeled cells are neuronal. In experiment B, both neuronal and epithelial cells are present. Briefly interpret and give an explanation for these results.

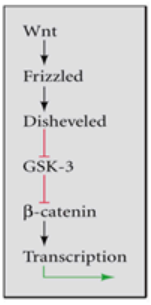
Use the following information to answer questions 7-9. In sea urchins, the micromeres, located on the bottom surface of the early embryo (as shown in a.) differentiate into mesoderm. When cultured alone, they still differentiate into mesoderm. You are investigating the role of β-catenin in this process. Below are some experiments that you have done:

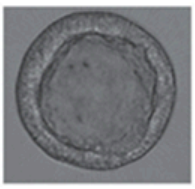


a. Early embryo labeled with an antibody to b-catenin

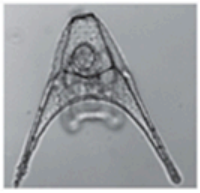


b. A "larva" that developed from an embryo treated with a GSK-3 inhibitor during early development: the entire embryo has differentiated into mesoderm





c. A "larva" that developed from an embryo injected with a dominant APC protein that binds constantly to b-catenin: entire embryo differentiates into ectoderm (no mesoderm)



d. A normal larva that has endoderm, mesoderm and ectoderm, and a recognizable structure

7. (.5) What do these experiments indicate about the role of β -catenin in the sea urchin embryo?
8. (.5) In which cells would you expect to see β-catenin expression in experiments b and c (all, none or some)?
9. (1) Given the results to experiments b. and c., describe which molecule APC could interact with in the pathway and how.