

Name: _____

2007 7.013 Problem Set 5

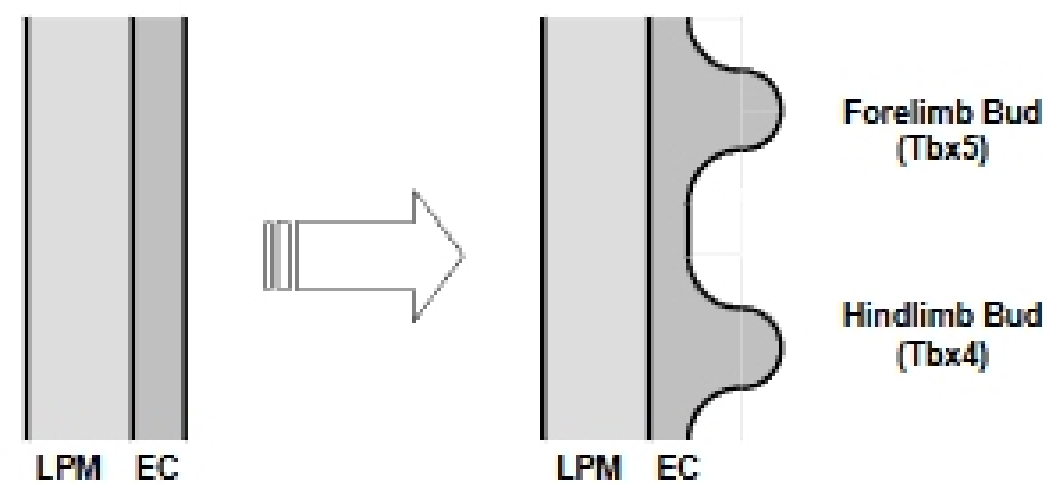
Due before 5 PM on WEDNESDAY, April 18, 2007.

Turn answers in to the box outside of 68-120.

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

Question 1.

The limb (arm or leg) forms when a group of cells called "lateral plate mesoderm" (LPM) induces overlying ectoderm (EC) to form an outgrowth called the "limb bud".



The limb bud forms an outgrowth because the number of cells it contains increases relative to surrounding ectoderm.

- 1a. One possibility is that cells in the bud undergo increased cell division relative to surrounding ectoderm. What technique could you use to ask whether this possibility is correct? (5 words or fewer)

BrdU labeling, which labels newly synthesized DNA. Or some other method that would mark dividing cells.

- 1b. Give another possible mechanism that would increase cell number. (5 words or fewer)

Decreased apoptosis in the limb bud, or increased apoptosis in the surrounding ectoderm.

- 1c. Interestingly, part of the brain (the "hindbrain") can substitute for the LPM and induce limb bud outgrowth. Discuss the molecular biology that is likely to explain this result (15 words or fewer).

The hindbrain and the LPM both produce some factor (maybe the same one) that is required for limb bud outgrowth (and hindbrain development).

- 1d. Young lateral ectoderm can give rise to many fates, including the limbs, brain and spinal cord, the epidermis of the skin, pigment cells. However, in older embryos, the lateral ectoderm can form only a limb bud. What is the term for the possible fates that a cell can form?

Potency or potential

Name: _____

The front and back legs of the chicken are very different, where the front limb is covered with feathers, and the hindlimb is covered with scales. In order to figure out why these limbs are different from one another, the following transplant experiment was performed. LPM from the hindlimb area was placed under future forelimb ectoderm, and LPM from the forelimb region was placed under future hindlimb ectoderm. The resulting chick embryos were assayed after the limbs had differentiated, and the following results were obtained.

future forelimb LPM	+	future forelimb ectoderm	→→	feathers
future hindlimb LPM	+	future forelimb ectoderm	→→	scales
future hindlimb LPM	+	future hindlimb ectoderm	→→	scales
future forelimb LPM	+	future hindlimb ectoderm	→→	feathers

- 1e. What do these data tell you regarding the mechanism by which fore- and hindlimbs make feathers or scales? (15 words or fewer)

Feathers or scales are induced by the LPM, not by the ectoderm.

Chickens have white feathers, quail have black feathers. You perform a transplant experiment like the one above, as detailed below, with the following results:

Chick forelimb LPM	+	chick ecto	→→→	white feathers
Quail forelimb LPM	+	chick ecto	→→→	white feathers
Chick forelimb LPM	+	quail ecto	→→→	black feathers
Quail forelimb LPM	+	quail ecto	→→→	black feathers

- 1f. What do these data tell you regarding the mechanism by which chick and quail make different color feathers? (15 words or fewer)

Feather color is dependent on the ectoderm, not on the LPM.

Name: _____

The Tbx4 transcription factor is expressed exclusively in the developing hindlimb, and is necessary for development of hindlimb characteristics, including whether it has scales or feathers. A mutant has been isolated where the hindlimb now looks like a forelimb.

Linkage analysis indicates that this mutant lies in the Tbx4 gene. However, in order to confirm mutant gene identity, you sequence the Tbx4 genomic DNA from this mutant, and find that there are no changes in any exon. On the other hand, you find two nucleotides in the mutant DNA, which differ from wild type. The first is at the exon1/intron1 splice acceptor sequence. The second is 25 bp 5' to the transcriptional start site, where the wild type sequence 5' TATAAAAT is instead 5'TATAGGGC.

- 1g. In the splice acceptor mutant, what changes would you observe in the RNA made from this gene, relative to wild type? (15 words or fewer)

The first intron would not be spliced out. This would result in a number of incorrect amino acids being added after exon 1, and probably an early stop codon or a frame shift.

- 1h. Could this change account for the Tbx4 mutant phenotype? Explain in 15 words or fewer.

Yes. There would be no functional Tbx4 translated from the aberrant RNA transcript.

- 1i. In the mutant that maps to the non-transcribed part of the gene (TATAAAAT), what changes would you observe in the RNA made from this gene, relative to wild type? (15 words or fewer).

TATA binding factor would not bind to the Tbx4 promoter, so RNA would not be transcribed.

- 1j. Could this change account for the mutant phenotype? Explain in 15 words or fewer.

Yes. There would be no Tbx4 transcript produced, and therefore no protein.

- 1k. Tbx5 is required for forelimb development, and normally expressed only in the forelimb. In the Tbx4 mutant, you find that Tbx5 is now expressed in both sets of limbs. Explain (use a diagram if you like).

Tbx4 --| Tbx5 → forelimb characteristics

Tbx5 promotes forelimb characteristics. Tbx4 inhibits Tbx5. With no Tbx4 in the mutant hindlimbs, Tbx5 is expressed there, resulting in forelimb characteristics.