

7.22 Fall 2005

sample exam 1 – KEY

7.22 Example Problems for Exam 1

The exam will be of this format. It will consist of 2-3 sets scenarios.

1. The Observation

During the first cleavage division of the nematode *Parascaris aequorum*, special cytoplasm, termed the germ plasm, is segregated into one specific daughter cell. Cells that do not inherit the germ plasm undergo a process called chromosome diminution (the chromosomes fragment and much of each chromosome is lost at subsequent divisions). All germ cells are descended from the cell that does inherit the germ plasm and these cells retain their full complement of DNA.

The Tasks

1.1 Offer a hypothesis about the molecular mechanism (involving, for example, a specific protein or RNA- feel free to give this(these) a name(s)) that might be responsible for the phenomenon (germ plasm vs chromosome fragmentation) described above.

A protein X, present in the cell that inherits the germ plasm, prevents chromosome fragmentation.

1.2a Describe an experiment to determine if the mechanism you have hypothesized and the phenomenon are correlated (correlation; “show it”).

Raise an antibody to protein X and do immunocytochemistry with this antibody on *Paracaris* embryos.

1.2b Describe a result that is consistent with your hypothesis.

Protein X localizes to the cell containing the germ plasm.

1.2c Describe a result that is inconsistent with your hypothesis.

Protein X does not localize to the cell containing the germ plasm.

1.3a Describe an experiment to determine if the mechanism you have hypothesized is necessary for the phenomenon to occur (loss-of-function; “block it”).

Produce a mutant worm that is deficient for protein X (or do RNAi against protein X prior to fertilization).

1.3b Describe a result that is consistent with your hypothesis.

The mutant has chromosome fragmentation in the cell containing the germ plasm.

1.3c Describe a result that is inconsistent with your hypothesis.

The mutant does not show chromosome fragmentation in the cell containing the germ plasm (i.e. the wild-type phenotype).

1.4a Describe an experiment to determine if the mechanism you have hypothesized is sufficient to the phenomenon to happen (gain-of-function; “move it”).

Inject RNA of the gene coding protein X into the somatic (i.e. non-germ

plasm inheriting) cells of the embryo.

1.4b Describe a result that is consistent with your hypothesis.

The injected worms do not fragment their chromosomes in the somatic cells of the embryo.

1.4c Describe a result that is inconsistent with your hypothesis.

The injected worms fragment their chromosomes normally in the somatic cells of the embryo.

2. The Observation

A key question in evolution and development is how the front (fore) limbs and back (hind) limbs become different from one another. That is, how their identity is established. One possible clue comes from expression of the Tbx5 transcription factor, which is normally expressed in the developing forelimbs, but not the developing hindlimb.

The Tasks

2.1 Formulate a hypothesis regarding the possible function of Tbx5 in directing limb identity.

Tbx5 activates expression of genes that produce the forelimb.

2.2a Describe an experiment to determine if Tbx5 is necessary for the function you have hypothesized (loss-of-function; “block it”).

Produce a mutant deficient for the Tbx5 gene.

2.2b Describe a result that is consistent with your hypothesis.

The Tbx5 deficient embryos have forelimbs that look like hindlimbs (or that look like neither hind- nor forelimbs).

2.2c Describe a result that is inconsistent with your hypothesis.

The Tbx5 deficient embryos have normal forelimb development.

2.3a Describe an experiment to determine if Tbx5 is sufficient to cause the function you have hypothesized (gain-of-function; “move it”).

Induce expression of Tbx5 in the developing hindlimbs.

2.3b Describe a result that is consistent with your hypothesis.

The injected embryos develop hindlimbs that look like forelimbs.

2.3c Describe a result that is inconsistent with your hypothesis.

The injected embryos develop normal hindlimbs.

3. The observation

In classic “transplant” experiments, the future neural plate (nervous system) of a frog embryo was moved from its usual dorsal position to the ventral region of a host embryo. The host and donor embryos were either at the early gastrula or late gastrula stage when the transplant was performed and all were examined when embryos reached mid neurula stage. Note: rewording!!

When the future neural plate of an early gastrula donor is transplanted, it develops as non-neural tissue (epidermis) on the ventral side of the host. However, when the future neural plate of a late gastrula donor is transplanted, it develops as a second neural plate on the ventral side of the host, as diagrammed below.

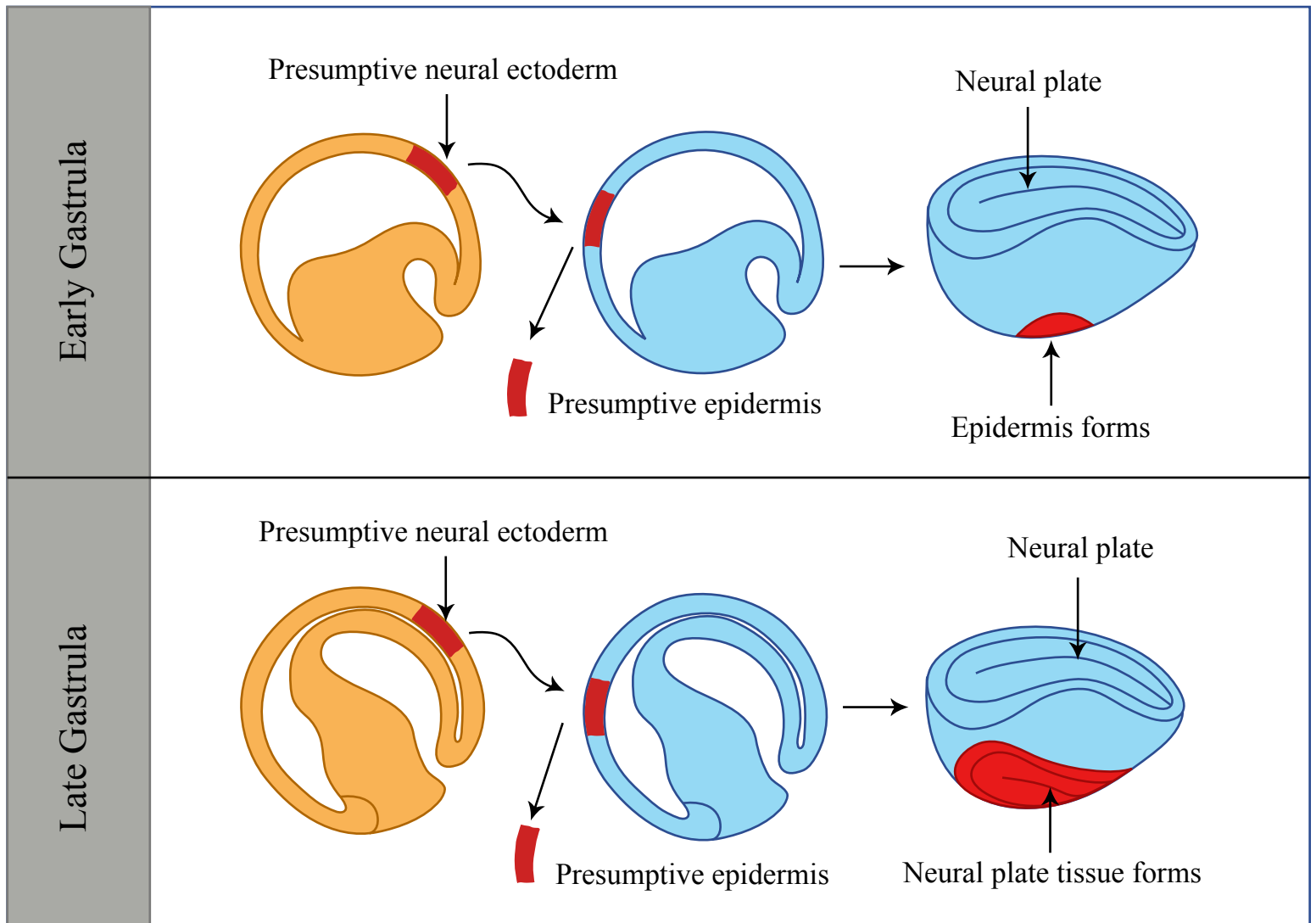


Figure by MIT OCW.

The Tasks

3.1 In order to perform this experiment, you need to know which piece of tissue is going to give rise to the normal neural plate, How would you determine this? What is this type of experiment called?

Inject a vital dye into the blastula stage embryo and ask later which become the neural plate. This is called a fate mapping experiment.

3.2 Formulate two possible hypotheses that would explain the results described.

- 1. The early gastrula epidermis inhibits the early gastrula neural ectoderm from forming a second neural plate, whereas the late gastrula epidermis had lost the ability to inhibit the late gastrula neural ectoderm from becoming a neural plate.**
- 2. The early gastrula neural ectoderm was not yet determined as neural plate, whereas the late gastrula neural ectoderm was determined as neural plate.**

3.3 The transcription factor NeuroD is expressed in late gastrula neural plate.

3.3a Describe an experiment to test correlation of NeuroD expression with the ability to form a neural plate (show it).

Perform *in situ* hybridization experiments on early and late gastrula stage embryos using a probe for NeuroD.

3.3b Describe a result consistent with this correlation.

NeuroD is not expressed in early stage gastrula, but is expressed in the late gastrula.

3.3c Describe a result inconsistent with this correlation.

NeuroD is expressed in both early and late gastrula stages, in the neural ectoderm. Note: This inconsistency would be stronger if one was monitoring protein expression by immunocytochemistry.

3.4a Describe a gain of function experiment to test function of NeuroD in neural plate formation (move it).

Inject RNA encoding NeuroD protein into the ventral side of an early embryo and ask whether you get ventral neural plate formation. Note: This will produce NeuroD where it is not normally made.

3.4b Describe a result consistent with this function.

The injected embryos form an extra neural plate on the ventral side.

3.4c Describe a result inconsistent with this function.

The injected embryos develop normally.

3.5a Describe a loss of function experiment to test function of NeuroD in neural plate formation (lose it).

Inject the dorsal sides of early embryos with an antisense oligonucleotide to NeuroD RNA.

3.5b Describe a result consistent with this function.

The injected embryos do not form a neural plate.

3.5c Describe a result inconsistent with this function.

The injected embryos develop normally.

4. The Observation

In an example we discussed in class, it was shown that while the animal half of an early sea urchin embryo does not form a normal pluteus larva, the micromeres can rescue development of the animal half. However, the micromeres contribute to only a few of the rescued embryos tissues.

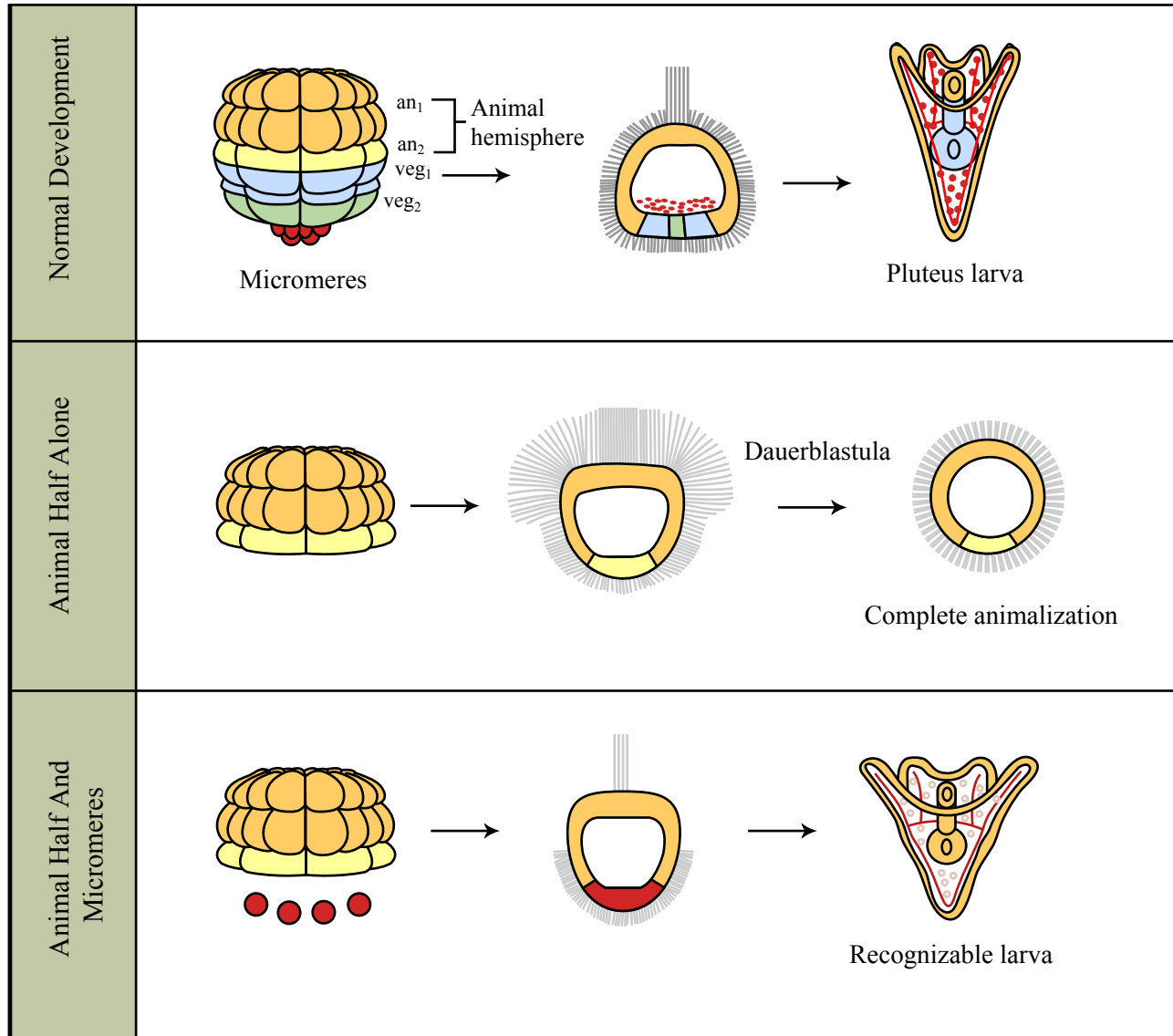


Figure by MIT OCW.

The Tasks

4.1 What major process does this observation illustrate?

Induction.

4.2 What is an “organizer”? Do you think this phenomenon could illustrate the activity of an organizer? Why?

A group of cells with profound inducing activity, that likely secrete multiple inducers. This could be an organizer because it is able to induce a large number of changes in the structure of the developing embryo.

4.3 The Delta protein is expressed in the micromeres of an intact sea urchin embryo. Delta is a secreted protein that acts on cells directly touching the secreting cell, via the Notch receptor.

4.3a Formulate a hypothesis concerning activity of Delta in the micromeres.

Delta is secreted by the micromeres and acts on the animal cells via the Notch receptor. The signal is then transduced to the nucleus and leads to transcription of factors that change the fate of the animal cells.

4.4a Describe an experiment to analyze correlation of Delta expression with the rescuing activity of the micromeres.

Use a Delta antibody to analyze the expression of Delta protein in the normal, defective and rescued embryos.

4.4b Describe a result consistent with your hypothesis.

Delta protein is expressed in micromeres of normal and rescued embryos, but not in the defective animal half embryos.

4.4c Describe a result inconsistent with your hypothesis.

Delta protein is expressed in the defective animal half embryos.

4.5a Describe an experiment to analyze necessity of Delta in rescuing activity of the micromeres.

Remove Delta protein from the precursors to the micromeres (using antisense oligonucleotides or a blocking antibody).

4.5b Describe a result consistent with your hypothesis.

The injected embryos look like defective animal half embryos.

4.5c Describe a result inconsistent with your hypothesis.

The injected embryos develop normally.

4.6a Describe an experiment to analyze sufficiency of Delta in rescuing activity of the micromeres.

Incubate the animal half embryos with purified Delta protein.

4.6b Describe a result consistent with your hypothesis.

The treated animal half embryos develop normally.

4.6c Describe a result inconsistent with your hypothesis.

The treated animal half embryos still form abnormal dauerblastulas.

5. First observation

During the early stages of mouse lung formation, a group of epithelial cells divides and forms “primary (1^o) tubules” which then branch and form additional secondary (2^o)

tubules, and eventually tertiary (3°) tubules. The epithelial cells touch a different cell type, the lung mesenchyme.

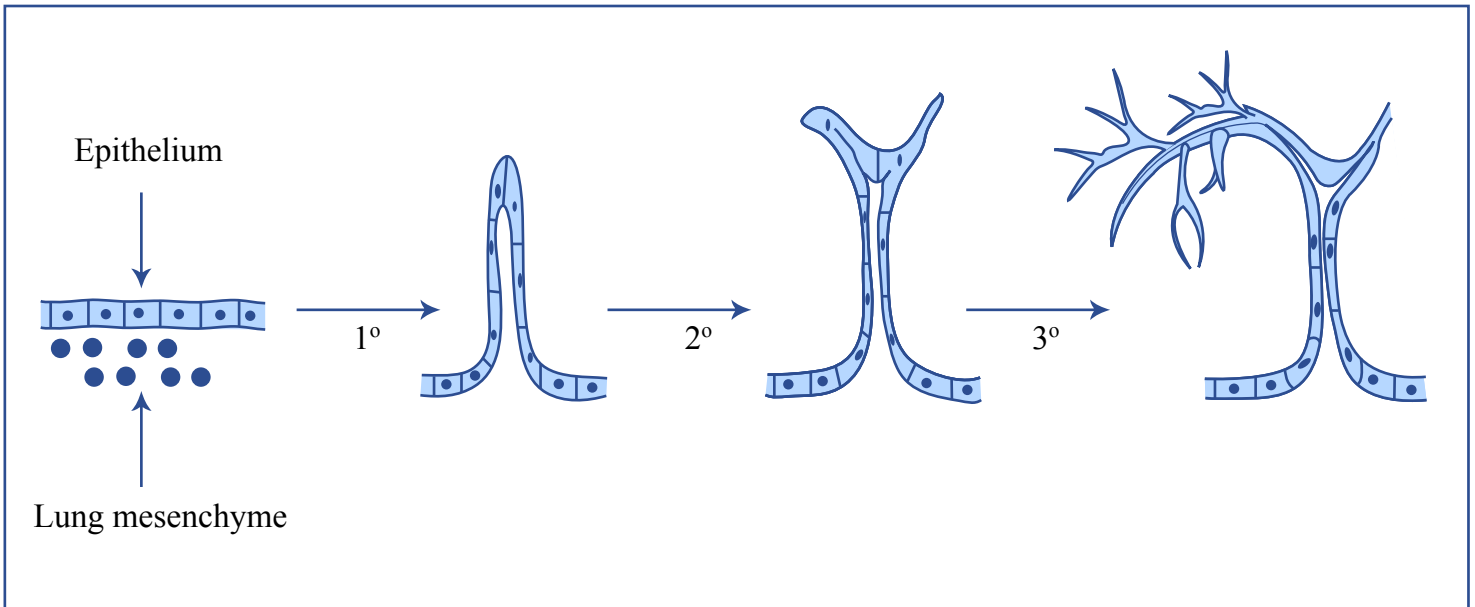


Figure by MIT OCW.

NOTE: Lung mesenchyme is present throughout the period of lung tubule formation

Second observation

The lung mesenchyme can be removed and/or substituted with kidney mesenchyme, as diagrammed below. In either case, tubules do not form.

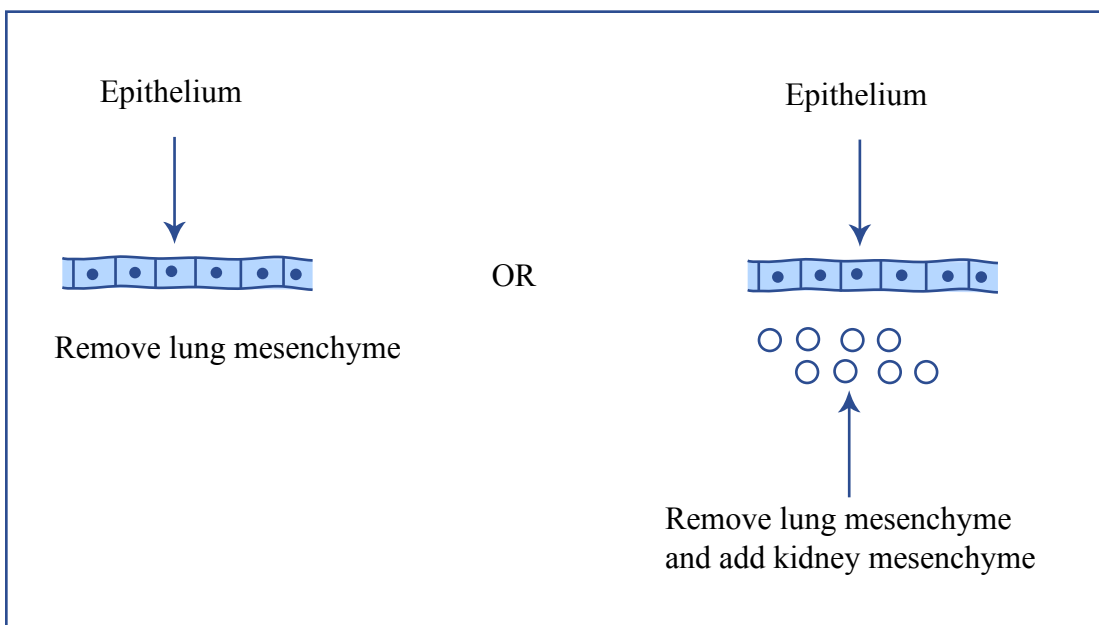


Figure by MIT OCW.

The tasks

- a. **10 points.** Formulate a hypothesis regarding the roles of epithelium and mesenchyme in primary lung tubule formation that takes the above observations into account.

The lung mesenchyme sends out a secreted signal that induces the epithelium to form tubules. There is a specific signal from the lung mesenchyme that cannot be substituted by the kidney mesenchyme.

- b. **1 point.** What fundamental process of developmental biology do these observations illustrate? (one word)

Induction

- c. **15 points.** The Fgf protein is expressed in the lung mesenchyme. Fgf is a secreted protein (a ligand) that acts through a receptor tyrosine kinase.

Describe an experiment to examine correlation of Fgf expression with lung tubule formation.

Develop an antibody to Fgf protein. Look at Fgf protein expression in developing lung and kidney tissue using immunocytochemistry.

Describe a result consistent with this correlation.

Fgf protein is expressed in lung mesenchyme, but not in kidney mesenchyme.

Describe a result inconsistent with this correlation.

Fgf protein is expressed in both lung and kidney mesenchyme.

- d. **15 points.** Loss of function tests:

Describe an experiment to test necessity (loss of function) of Fgf for lung tubule formation.

Create a mouse knockout of the Fgf gene, thus blocking production of the Fgf protein.

Describe a result consistent with necessity.

Fgf knockout mice do not form lung tubules.

Describe a result inconsistent with necessity.

Fgf knockout mice have normal lung tubule formation.

- e. **15 points.** Gain of function tests:

Describe an experiment to test sufficiency (gain of function) of Fgf in lung tubule formation.

Incubate the lung epithelium with purified Fgf protein.

Describe a result consistent with sufficiency.

Lung epithelium incubated with purified Fgf protein forms lung tubules.

Describe a result inconsistent with sufficiency.

Lung epithelium incubated with purified Fgf protein does not form lung tubules.

- f. **10 points.** Where might you expect the Fgf receptor protein to be localized? Include the cell types and expected subcellular localization.
On the plasma membranes of lung epithelial cells.

How would you test this expectation?

Produce an antibody against the Fgf receptor. Perform immunocytochemistry on lung tissue to determine where the receptor is localized.

- g. **10 points.** While Fgf promotes lung tubule outgrowth, the lung mesenchyme also produces the secreted Sprouty protein, that inhibits outgrowth of the primary lung tubules. The dual action of Fgf and Sprouty therefore lead to primary tubules of the correct length.

Formulate a hypothesis regarding the molecular mechanism by which Sprouty and Fgf might function together to regulate primary lung tubule formation.

A high level of secreted Fgf protein activates expression of Sprouty.

6. The observations

In vertebrates there are at least 19 different Fgf ligands and 4 different Fgf receptors! Fgf8 is expressed in the developing hindbrain, that will give rise to the medulla and cerebellum. Fgf8 is essential for normal hindbrain development. Fgf8 is also expressed in the developing limbs, where it is essential for normal limb development.

The tasks

- a. **10 points.** Limb and hindbrain have very different functions. In what general ways is hindbrain different from limb? Give an answer that uses your knowledge of cell and molecular biology.

The cell types are different, because they express different sets of gene products.

- b. **1 point.** The limb develops primarily from mesoderm, the hindbrain from ectoderm. What is the developmental biological term that describes collectively the ectoderm, mesoderm and endoderm? (two words)

Germ layers

- c. **15 points.** If pure Fgf8 protein is applied to isolated ectoderm, the ectoderm goes on to form a rudimentary hindbrain. If Fgf8 protein is applied to isolated mesoderm, the mesoderm goes on to form the beginnings of limbs.

Give the developmental biological term that describes differences in the ability of a tissue to respond to the same stimulus. (one word)

Competence

Formulate a hypothesis regarding the molecular mechanism by which the ectoderm and mesoderm respond differently to applied Fgf8 protein.

Fgf8 binds to an Fgf receptor and the signal is transduced to the nucleus. In the nucleus the transcription factors activated by the Fgf signal interact with different co-factors that are present in ectoderm or mesoderm, and thus turn on different down-stream genes.