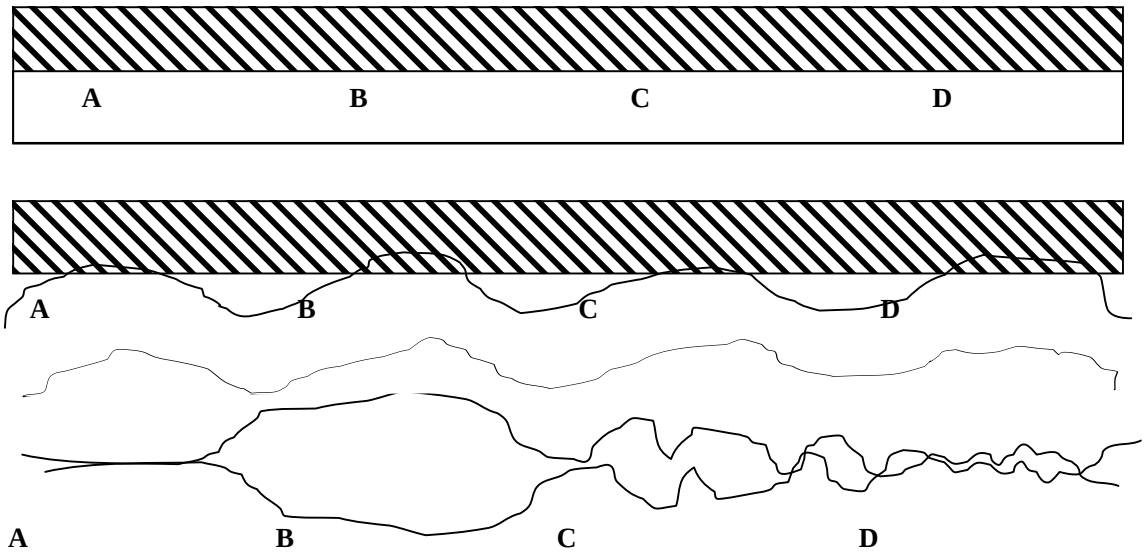
Key: 7.013 Recitation 14 – Spring 2018

1. This question is about development of the alimentary canal (the "gut") from the "endodermal" cell layer of the mouse embryo. This process requires contact between the endoderm (unshaded, labeled A, B, C, D in the diagrams below) and adjacent "mesodermal" cell layer (striped). Initially, a tube develops in the endoderm of the embryo. A few days later, the tube becomes kinked, and three days after that, four obvious morphological divisions (A, B, C, D) can be seen along the antero-posterior length of the tube, as diagrammed below. A, B, C, and D correspond the same regions at all stages (that will give rise to the esophagus, stomach, small intestine, large intestine, respectively). Differentiation occurs only at the latest stage, 1 week after the straight tube stage.

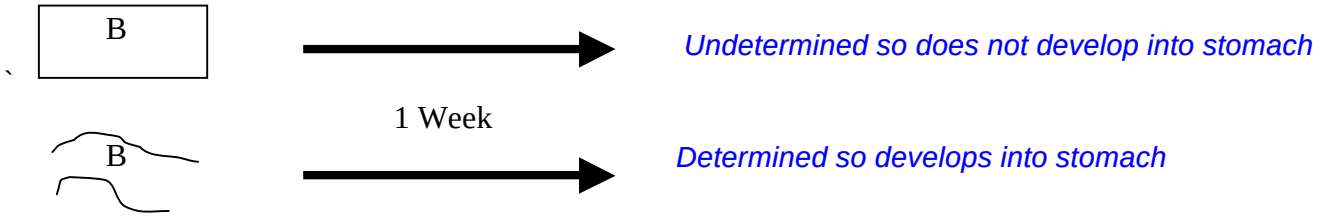


In order to analyze the timing of stomach determination, you do the following transplant experiment within the whole embryo in which you invert the endoderm of regions A and B (the future esophagus and stomach) at either straight or kinked tube stage, as indicated below. The mesoderm is left intact and is not inverted. After the gut regions have differentiated, you observe that in the straight tube transplant, a normal gut develops, whereas in the kinked tube stage transplant, the position of the esophagus and stomach are inverted.

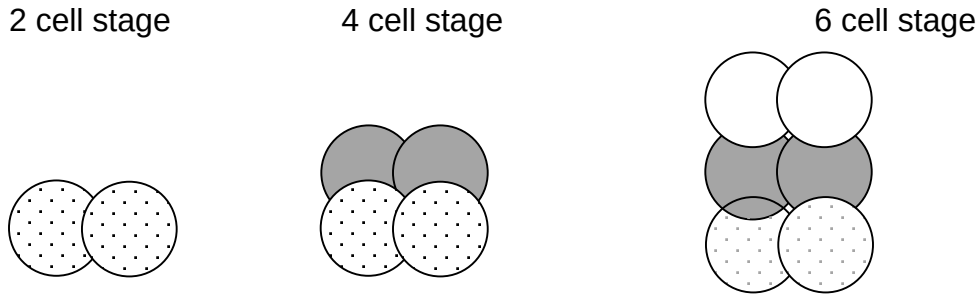
a) Distinguish between "determination" and "differentiation."
Determination refers to the decision to become a certain cell type, whereas differentiation refers to formation of the final functional cell.

b) When does stomach determination occur? **Explain**.
At or just before the kinked tube stage, since at this time, the fate of the endoderm is fixed and cannot be altered by adjacent mesoderm. The first result, at the straight tube stage implies that the mesoderm is responsible for instructing (inducing) specific regions of the gut to develop. By the kinked tube stage, the identity of the stomach is fixed.

c) In a second set of experiments, you isolate (explant) the endoderm of region B at both the straight and kinked tube stages, and then culture it for one week **without the mesoderm**. Based on the results of the transplant assay above, what would you predict the outcome would be?



2. Leeches are medicinally important animals as they produce hirudin, a very effective anti-clotting agent. As embryos, they undergo interesting cell division, where a new row of cells is added at every division, so 1st row cells are the oldest cells, 2nd row cells are produced from division of 1st row cells, and 3rd row cells are produced from division of 2nd row cells. Based on patterns of gene expression, the following different "territories" (each a precursor to specific cell fates) are observed at each division.



a) Formulate a hypothesis, based on segregation of cell autonomous regulatory factors to account for the change in number of territories from the 2 cell to the 4 cell stage. What is the term for this type of regulatory factors?

The 1st row cells retain a determinant that regulates their specific fate. The 2nd row cells do not receive this and therefore are different from 1st row cells. Conversely, you could hypothesize that the 2nd row cells inherit a determinant at cell division that is not retained in the 1st row cells, and therefore their fates are different. Thus depending upon the receipt of the determinant different cells can end up having different fates.

b) Formulate a hypothesis, based on cell-cell signaling to account for the change in number of territories from the 4 cell to the 6 cell stage. Comment on when this signaling is likely to occur. What is the term for this type of signaling?

2nd row cells secrete a ligand that acts on 3rd row cells to change their fate. The 3rd row cells contain the receptor and downstream signaling components for this ligand. This is called induction

3. Since liver contains detoxifying enzymes, there is a great interest in understanding liver organogenesis. Hybrid/bionic livers to date consist of a suspension of hepatocytes (liver cells) on a synthetic support. However, the bionic livers have limited use, as the hepatocytes stop functioning 2 days after being added to the device.

a) You hypothesize that hepatocyte function may be prolonged by addition of certain signaling molecules. You test the following combinations of ligands (*BMP, Fgf, Shh &Wnt8*) to see if they prolong hepatocyte function in the bionic livers and obtain the following results.

Factor	Time (days) for which hepatocytes are functional
Control hepatocytes (with no factor)	2
BMP+Wnt8+Fgf + Shh	6
BMP+Wnt8+Fgf	6
BMP+Fgf+Shh	1
Wnt8+Fgf+Shh	6

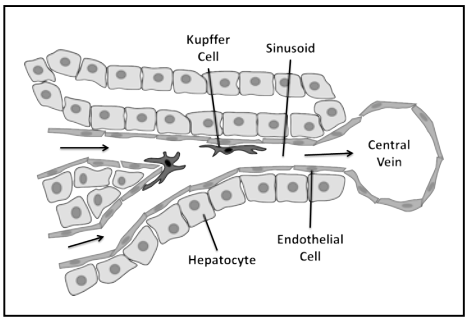
iii. Which ligand(s) is most important in prolonging the liver function? **Explain** why you selected this option.

Wnt 8 *appears to be essential either alone or in combination with Fgf. One may assay wnt8 alone, compared to wnt8+Fgf, to determine whether Fgf is required.*

iv. What activity does shh have on hepatocyte function*?*

It is an inhibitor in the absence of Wnt+Fgf, which can overcome its inhibitory effect.

b) The following is a schematic of liver organogenesis during embryonic development.



If a ligand you have identified normally regulates liver function, where would you expect to observe expression of ….

iii. This ligand (*choose from hepatocytes, endothelial cells, kupfer cells and sinusoids*)?

iv. The receptor for this ligand (*choose from hepatocytes, endothelial cells, kupfer cells and sinusoids*)?

c) The sinusoids are tubes that arise from single cells that associate to form a sheet, which eventually forms a tube. What would be the effect of each of the following perturbations on the formation of sinusoids?

Perturbation	Sinusoid tube formed (*Yes/ No*)? **Explain** your choice.
Loss- of- epithelial apical/ basal polarity	*No, this perturbation will cause the cells to undergo an epithelial to mesenchymal transition (EMT). The cells in mesenchymal state will not be able to attach and form sinusoids.*
Actin depolymerization	*No. Actin is a cytoskeletal protein that exists in the form of G monomers that can polymerize to form F actin polymer. Its depolymerization will alter cell shape and convert the cells from the EMT state, which allows them to migrate.*

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