# 7.013 Recitation 14 – Spring 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

## Summary of Lectures 24 (4/13) and 25 (4/18)

Development is the process of forming a multi cellular organism from a single fertilized egg. This involves a balance between cell division and cell death, formation of cell types through cell differentiation, positioning different cell types at the correct locations, and organizing cells into the correct three-dimensional shapes to form tissues, organ, systems and hence the whole organism.

All cells in an organism have the same set of genes, yet different cell types express different sets of genes (combinatorial codes) which regulate which cell type will form. The functional form of a cell is called its “fate”. Cells make stepwise decisions to assume their fate. Undecided cells are termed “uncommitted” or “undetermined”. These cells become determined or committed, once they have decided their fate, but have not yet assumed it. Subsequently, cells differentiate to assume their final fate. Determination involves activation of a few regulatory genes, which control activation of many effector genes in the differentiated cells. The regulatory gene, effector genes and the ubiquitously expressed genes together constitute the combinatorial code of a cell type, which distinguishes it from other cell– types. The combinatorial code of a cell type is expressed step-wise.

As development proceeds, the number of possible cell types produced by a cell gets reduced. Or in other words, the potency (number of fates of a cell type) reduces over time during development. The number of cell types originating from a single cell is referred to as the lineage.

Cells become different through cell/cell signaling (induction) and asymmetric localization of regulatory molecules (morphogens) that include transcription factors and translation factors and secreted ligands. If the morphogen acts in a cell autonomous fashion (it is produced by a cell and acts within the cell) it is referred to as a determinant. In comparison, if a morphogen is produced and secreted by a cell and it binds to a receptor on the surface of another cell to regulate its fate, it is called an inducer. Morphogens (both determinants and inducers) by definition act in a concentration dependent manner to regulate cell- fate.These are located in the “organizer”, which may be defined as the group of cells many signaling pathways ligands. These cells and their ligands have an enormous influence on the surrounding cells.

In animal development, a key step is the initial generation of asymmetry, which can occur before, at, or after fertilization depending on the type of species. This results in partitioning of one or more regulatory molecules to just part of the early embryo. Development of multi-cellular organisms proceeds in steps, with increasing complexity over time. The initial cell divisions in a developing embryo are rapid and synchronous. However, as cell division proceeds, further asymmetry is generated through induction and/or localization of determinants, inducers or chromatin modifications etc. resulting ultimately in the determination and differentiation of various cell types. The number of fate options available to a cell (potency) decreases with time. The zygote is totipotent, embryonic cells are multi- potent/pluripotent and older cells are generally uni- potent or bi- potent. In adults you see hematopoetic stem cells (HSCs) that are multi- potent and neurons, which is terminally differentiated. The mature Red blood cells (RBCs) in us do not have nuclei at all!

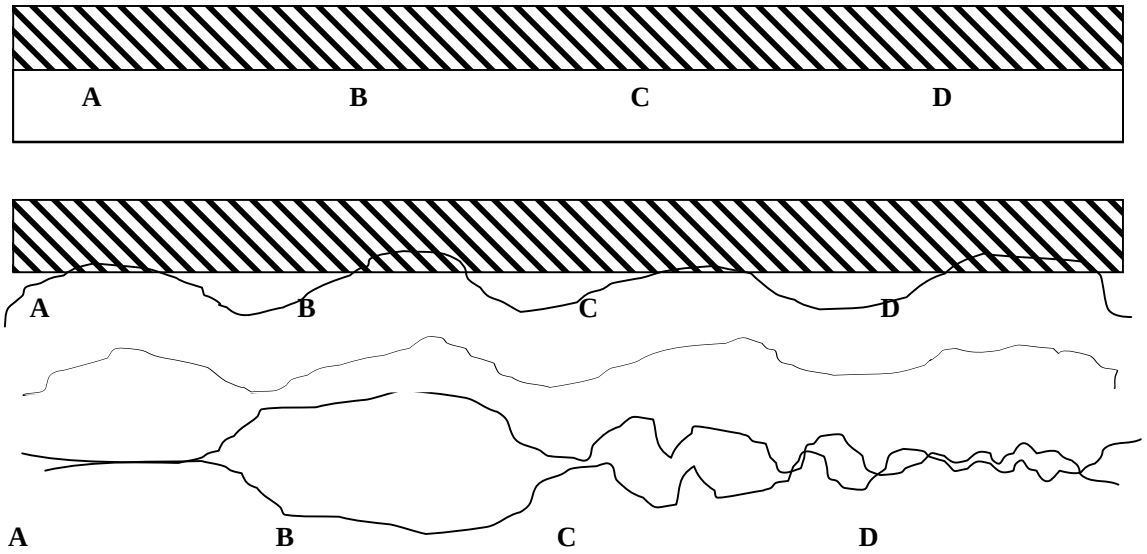
The embryonic axes (dorsoventral, anteroposterior and left/right) are generated very early during development, in some animals by the two or four cell stage. The stages of development are given different names: blastula is a ball of cells formed during the first set of cell divisions after fertilization, gastrula stage occurs subsequently and in this stage the cells begin to move, neurula and later stages include the onset of differentiation. At gastrula, the first cell types can be distinguished, although these are not differentiated. They are called ectoderm, which is on the outside of the embryo and will eventually form the nervous system and outer, epidermal layer of the skin; the mesoderm which will

form the heart, kidneys, blood, gonads and other tissues; and the endoderm, on the inside of the embryo, which will form the lungs and digestive system (gut).

**Morphogenesis& Organ development** Morphogenesis is the process by which cells and organs aquire the 3D structure that is essential for their function. Processes that direct morphogenesis are cell sorting, adhesion, movement, shape change, division, death and responsiveness to positional signals. Two general arrangements of cells are found. Mesenchymal cells are single cells that can migrate and change shape, and which lie on the extracellular matrix (ECM). Epithelia are sheets of cells that are held together by belts of tight junctions, puncta of adherens junctions and calcium-dependent adhesion molecules. The latter are responsible for homotypic (between like cells) adhesion. Mesenchymal and epithelial states can interchange in the epithelial/mesenchymal transition (EMT), or vice versa (the MET). An apical and basal (or basolateral) membrane can be distinguished on epithelia, which contain different proteins. Epithelia also lie on an ECM, often called the basement membrane, which is a meshwork of glycoproteins. The cytoskeleton is pivotal in changing the shape of cells, and in migration. Polymerization of microtubules and microfilaments is regulated by cell signaling, often arising from ligands in the ECM that bind receptors on the cell surface. Transition of G-actin (unpolymerized) to F-actin (polymerized) is especially important. Tube formation is pivotal in morphogenesis. Epithelial sheets can roll to form tubes, or the sheet can extend, through both cell division and cell elongation. Mesenchymal cells can condense and then form epithelial tubes. Single cells can wrap or hollow out to form tiny tubes. Both tube length and diameter are regulated.

## Key

**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

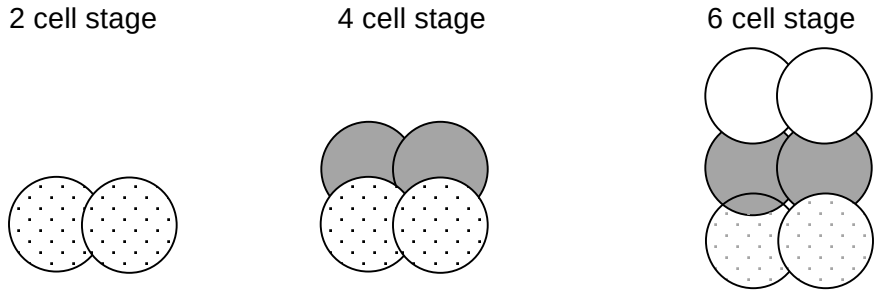
and is not inverted. After the gut regions have differentiated, you observe that in the straight tube transplant, a normal gut develops, whereas in the kinked tube stage transplant, the position of the esophagus and stomach are inverted.

**a)** Distinguish between "determination" and "differentiation."

**b)** When does stomach determination occur? **Explain**.

**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?

**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?

**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.

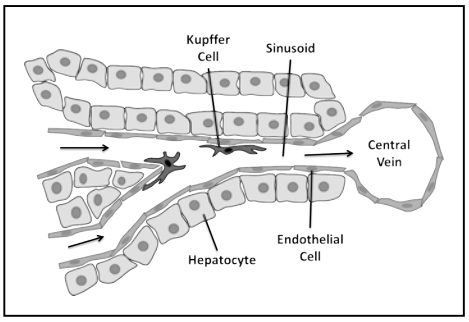
**a)** You hypothesize that hepatocyte function may be prolonged by addition of certain signaling molecules. You test the following combinations of ligands (*BMP, Fgf, Shh &Wnt8*) to see if they prolong hepatocyte function in the bionic livers and obtain the following results.

| 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 |

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

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

**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 ….

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

**ii.** 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 | |
| Actin depolymerization | |

# 7.013 Introductory Biology

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