Lecture 5

Gastrulation

5. The dorsal/ventral axis in the frog
   a. Mother sets up initial axis of polarity: animal & vegetal poles
   b. sperm entry is the critical cue in setting up the dorsal-ventral axis and results
      in formation of the blastopore 180° opposite sperm entry point.  How does
      this work?
   c. Sperm enters randomly at one side of the animal hemisphere
      1.) Entry of sperm nucleus leads to cytoskeletal rearrangements which lead to
         a rotation of outer cortex relative to inner cytoplasm.
      2.) This rotation leads to juxtaposition of animal pole cytoplasm with vegetal pole
         cortex, and forms an area called the gray crescent.  The gray crescent region in
         the fertilized egg marks the future site of the dorsal blastopore lip in the later
         embryo starting to undergo gastrulation.

6. Experiments in Gastrulation
   a. Experiment #1: determine basis of gray crescent formation
      i. Rotate egg so that sperm entry point is now on top.
      ii. Prevents normal 30° cortical rotation.
      iii. As a result:
          1. Inner cytoplasm shifts due to effects of gravity
          2. New region of juxtaposition of animal pole cytoplasm -- vegetal pole
             cortex next to sperm entry point
          3. Blastopore forms there instead.
      Conclusion: juxtaposition of animal pole cytoplasm to vegetal pole cortex
      is critical factor!

Induction

1) In the first lecture, we talked about how cells can get information from
   neighbors which can influence their cell fate.  Today we'll be talking more in
   detail about the kind of signaling that can communicate information between
   cells.

   a) INDUCTION is the process whereby one group of cells provides information to a
      second group of cells, and this information specifies or influences the FATE of the
      second group of cells.
   b) To have an induction we need two components:
      i) an INDUCER, the cells that produce a signal (ie. small protein or ligand) that will
         change the behavior of other cells.
      ii) a RESPONDER, the cells that respond to the signal from the inducer.
   c) Not all cells can respond to signals from the inducer: the ability to respond to a
      certain inductive signal=COMPETENCE.  The responder cells must be competent to
      receive the inducing signal (ie: express a receptor)
   d) By successive inductions it is possible to generate many different cell types from a
      few interactions.  Ie. induced cells (cells that were responders) that have made a cell
fate decision can turn around and induce other tissues, or even the cells that were the original inducers.

e) The cell movements involved in gastrulation provide cells with many new neighbors and thus many opportunities for inductions.

2) Review of different types of signaling and signaling molecules.
   a) Signaling molecules can be proteins or small molecules
   b) Signals can act
      i) Very short range- cells in direct contact.
      ii) Locally among neighbors, i.e. by secreted molecules that travel a short distance.
      iii) Globally throughout the body. I.e. hormones produced by pituitary act in the ovary on developing follicles.
      iv) In a graded fashion. I.e. strong signal close by, weaker signal farther away. If high levels of signal, get one cell fate. If low levels, get a different cell fate.
   c) Induction allows an initial difference to be amplified into many cell types

**Gastrulation and Induction**

1. Experiments in Gastrulation (continued)

   b. Experiment #2—Dorsal blastopore lip is the organizer.
      most famous transplantation experiment that resulted in a Nobel prize. Hans Spemann and Hilde Mangold published this in 1924 from Mangold’s doctoral thesis. Transplant dorsal blastopore lip of newt embryo (forms at gray crescent region) to another region of recipient embryo. Causes the formation of two blastopores and a double embryo. They named this region the Organizer, since it can direct formation of gastrulation and an entire new axis.
   
   c. Experiment #3—Induction of dorsal blastopore lip in Xenopus.
      1. Start with 2 embryos, one normal, one irradiated (UV irradiation prevents gastrulation). Transplant dorsal vegetal blastomeres (which underlie the prospective dorsal lip region) of normal embryo into irradiated embryo. In an irradiated embryo that didn’t undergo transplantation, would just get ventral ‘belly piece’ since gastrulation failed to occur. If do transplant, get normal embryo.
      2. If transplant dorsal vegetal blastomeres of normal embryo into ventral side of another normal embryo, get a 2nd blastopore and axis in addition to original.
      Conclusion: Dorsal vegetal cells underlying the prospective dorsal lip induce them to initiate gastrulation.

2. MESODERM INDUCTION in the frog embryo (example of local signaling).
   Initial asymmetries of *Xenopus* eggs (e.g. animal & vegetal poles) lead to some cell fate differences, but not all fates can be created this way! These cell fates arise from cell:cell interactions. E.g. Mesoderm arises at ectoderm/endoderm junction. Top half (animal pole) of frog blastula is fated to become ectoderm (top) and mesoderm (equatorial part), while bottom half (vegetal pole) is fated to become endoderm. We’re going to go over a few experiments that demonstrated how induction of the mesoderm occurs:
   
   i. Experiments: Neither vegetal nor animal pole alone can make mesoderm.
1. If you separate animal (animal caps) and vegetal parts and culture them separately
Result: Vegetal pole cells become endoderm and Animal pole cells become ectoderm, but no mesoderm forms. [If you isolate animal cells that were located in the marginal zone and did come in contact with vegetal cells before you removed them, they can make mesoderm when cultured separately]

2. **If you culture animal cap and vegetal cells together**, vegetal cells still form endoderm, but the animal caps can be induced to form mesoderm instead of ectoderm. **Thus, vegetal pole cells can induce animal pole cells to make mesoderm**

3. **THEREFORE A SIGNAL IN THE VEGETAL PART IS NEEDED TO INDUCE MESODERM IN THE ANIMAL CAPS.**

   ii. **Experiment: regional specificity of dorsal mesoderm induction**
   (dorsalmost vegetal cells induce formation of dorsal mesoderm in cell above them)

   iii. These and other experiments led to the **3-step MODEL OF MESODERM INDUCTION**.
   1. Dorsal-most vegetal cells induce dorsal animal pole cells above them to become the "organizer" (and also dorsal mesoderm). [These dorsal vegetal cells able to induce formation of the dorsal mesoderm were named the Nieuwkoop center after a Dutch scientist Pieter Nieuwkoop who was involved in these experiments in the 1960s and 70s.]
   2. More ventral vegetal cells induce other animal pole cells to become ventral + intermediate mesoderm
   3. The Organizer (dorsal margin cells) induces the neighboring margin zone cells to become intermediate mesoderm.

   iv. **What are the molecular pathways responsible for mesoderm induction?**