Lecture 7

Development of the Fruit Fly *Drosophila*

1. **The fruit fly- a highly successful, specialized organism**
   a. Quick life cycle includes three larval stages and a metamorphosis. **Spends only 1 day as an embryo**
   b. Body plan built from a repeated set of units: segments. Sets of genes control development in segmented animals (including us) by specifying and refining segment fates.
      i. Each segment has unique identity!
      iii. True for maggot and adult.

2. **Why flies?**
   a. Lots of reasons we talked about the first day of class: small, sequenced genome, many genetic and molecular tools, small and cheap.
   b. Very reproducible anatomy- every hair same from one fly to next, and can see segmentation on outside of body
   c. Anatomical, developmental, & even behavioral similarities to vertebrates. Adult flies do many things that we do - they eat, sleep, learn, have sex etc. So these processes can be genetically analyzed in flies.
   d. Primary reason- **History**
      i. In early 1900s when science of genetics rediscovered, Thomas Hunt Morgan chose fruit fly to study genetics. He was one of first scientists to systematically isolate "mutations" affecting visible traits (at first just found naturally occuring mutations). A “mutant” is an animal lacking the product of single gene. The first fly mutant found in 1910 was the white mutant.
         1. Normal, wild-type flies have eyes containing red pigments that protect vision.
         2. Found mutant fly with white eyes.
         3. White gene encodes a transmembrane channel protein that pumps pigment precursors into cells. It turns out that it’s a member of a large family of channel proteins.
         4. Related to a human gene that when mutated causes cystic fibrosis! This related CFTR protein is a transmembrane channel protein that moves chloride ions.
      ii. Thousands of mutations have now been identified that affect all aspects of body structure/function. Christiane Nüsslein-Volhard and Eric Wieschaus set up a huge screen to look for developmental mutants that affect the body plan of fruit flies - they got lots of mutants and a Nobel prize! The analysis of these mutations has increased our knowledge of development dramatically.
   e. Today, we’re going to talk about some of the keys stages of embryonic development and pattern formation in the fly—remember that the fly’s body plan is assembled in 24 hours!
3. **The Maternal Effect Genes Establish the Anterior-Posterior axis early in development.**
   a. These genes, like bicoid and nanos, are mRNAs made by nurse cells and pumped into the oocyte through cytoplasmic bridges. They form gradients which set up the Anterior-Posterior axis. The proteins are mostly transcription factors that activate **GAP GENES** (see below).
   b. Mutations in these genes are called maternal effect mutants because the genotype of the mother determines the phenotype of the offspring.

4. **Let’s think about one of these maternal effect genes, bicoid, as an example.**
   a. bicoid mRNA localized to anterior end. Upon fertilization mRNA is translated into protein which forms a gradient—lots of Bicoid at anterior end, some in middle, and none at posterior end. bicoid encodes transcription factor- i.e. it turns other genes on or off.
      i. Remember, these proteins and RNAs are used by the embryo during cleavage, when nuclei are dividing but cell membranes haven’t yet formed. Therefore, gradients can form easily.
   b. **Different genes are activated by different levels of Bicoid**
      i. Some genes only turned on where there is lots of Bicoid.
      ii. Other genes only turned on by moderate amounts of Bicoid
      iii. Other genes turned off by Bicoid--therefore only turned on where bicoid is absent.
      iv. Thus this gradient of Bicoid leads to the next set of genes being activated in broad regions of the embryo. These expression domains, in turn, can interact to define new subdivisions
   c. **How do we know this?**
      i. **Experiment #1-** Remove Bicoid from embryo i.e. bicoid mutant embryo
         1. **In absence of Bicoid anterior region of body missing** (embryo has no head and thorax).
      ii. **Gap genes** regulated by bicoid also encode transcription factors e.g. Hunchback, Kruppel, Knirps.
         a. Hunchback is expressed in anterior region where high bicoid.
         b. Kruppel is expressed in central region of embryo where moderate bicoid
         c. Knirps expressed in posterior stripe where less bicoid.
         d. Hunchback maternal mRNA distributed throughout oocyte, but its translation is inhibited in posterior end AND additional transcription of Hunchback stimulated by bicoid...so its both a maternal effect gene and a gap gene

5. **The GAP GENES Set Up the First Crude Embryonic Subdivisions**
   a. Gap genes initially identified through genetics. **Embryos mutant for a particular gap gene are missing entire regions of the body plan that match where that gap gene is expressed.** Ie. kruppel mutant missing thorax segments and part of the abdomen.
   b. Gap gene proteins then turn on further downstream genes (the PAIR-RULE GENES).
      i. Some genes are turned on by hunchback -- some genes by Kruppel
      ii. Other genes are turned on only where both proteins present (where they overlap)

6. **The PAIR-RULE GENES**, such as **Even-skipped**, **help the embryo form more refined segments**. Most pair rule genes are expressed in 7 stripes or every other parasegment (**Parasegments** are found in the embryo, later larval and adult stages have **segments**
which are built on the basis of the parasegment patterning ie. a segment contains parts of 2 parasegments. See Figs 9.27 and 9.33). Most pair-rule genes encode transcription factors that control expression of **SEGMENT POLARITY GENES** like *engrailed* and *wingless* that we’ve talked about previously.

7. **The Segment Polarity genes define anterior/posterior regions of segment**
   a. Remember *wingless* and *engrailed* define the anterior vs. posterior of segments, by specifying which cells will have a posterior (hairless) fate.
      i. *wingless* expressed only in third cell of each segment
      ii. *engrailed* only expressed in fourth cell of each segment
   b. For example, *engrailed* is activated in cells that have high levels of the pair-rule transcription factors *Even-skipped* or *Fushi tarazu*. *Wingless* is activated in cells that receive no *Even-skipped* or *Fushi tarazu* but contain another pair-rule protein not shown here—*Sloppy-paired*.
   c. Some of the segment polarity genes like *engrailed* encode transcription factors, but others encode secreted signals, like *wingless*. The segment polarity genes and the pair-rule genes control expression of **homeotic genes**.

8. **The homeotic genes: how to tell your head from your ...abdomen**
   a. Dividing the embryo into segments is only part of establishing the body plan. As mentioned above, the segment polarity genes, like *wingless* and *engrailed*, set up an anterior-posterior pattern within segments. However, if nothing else happens all segments in the body would be identical!
   b. **The HOMEOTIC GENES determine segment identity.** They were first identified by mutations that caused one body part to turn into another. The *antennapedia* mutant is a classic example of a homeotic mutant. See legs in place of antenna! [This is a special **gain-of-function** mutation (in this mutant Antennapedia gene is expressed in head region where it is normally not expressed in wildtype flies). Normally, Antennapedia is only expressed in thoracic region. **Loss-of-function** antennapedia mutants have antenna in place of 2nd set of legs. ie. T2 fate is not correctly specified.]
   c. The person who pioneered studying the homeotic genes in flies is **Ed Lewis**, and he shared the 1995 Nobel prize with Nüsslein-Volhard and Wieschaus. He wanted to understand how genes regulate the identity of specific structures.
   d. For instance, as we see in wildtype flies, a pair of legs is only made by each of the three thoracic segments not by the head segments. And only the second thoracic segment makes a pair of wings plus a pair of legs. How is this controlled?
   e. Lewis looked a lot at mutations in the homeotic gene *ultrabithorax*. In one type of ultrabithorax mutant (partial loss-of-function: *Ubx* just lost in T3), the 3rd thoracic segment (which normally doesn’t make wings) takes on the identity of the 2nd thoracic segment, resulting in a **fly with 4 wings**. Lewis’s conclusion from his genetic analysis: T2 is the “ground state”, and Ultrabithorax is the master regulator of 3rd thoracic identity.
      i. Ultrabithorax encodes a transcription factor. **Hypothesis:** *Ubx* is the **Master regulator that turns on T3 and A1 specific genes**.
         1. Experiment #1- Is Ultrabithorax expressed in right place at right time? Yes- expressed in T3 and A1 at time when segmental identity being established.
         2. Experiment #2- Can Ultrabithorax protein bind to DNA and regulate genes? Yes- e.g. Turns on gene required to make legs.
   f. One of the stunning discoveries that came out of studying flies is that there is a set of homeotic genes including ultrabithorax and antennapedia, each controlling the identity of part of the body.
g. These homeotic genes all encode transcription factors, with a conserved DNA binding HOMEODOMAIN. They are found in 2 major clusters - the Antennapedia (ANT-C) and the bithorax (BX-C) complexes. ANT-C has 5 genes and BX-C has 3 genes. In one of the most bizarre mysteries in modern genetics, the order of the homeotic genes on the chromosome matches the order of the embryonic regions that they specify, from anterior to posterior.

h. Another key point about homeotic genes is that each homeotic gene is negatively regulated by the homeotic gene products expressed posterior to it. Ie. all of the genes in the bithorax complex—Ultrabithorax, Abdominal A, and Abdominal B (which are master regulators of the abdomen!)—repress the expression of Antennapedia. So if this entire complex of 3 genes is deleted, Antennapedia expression will extend throughout the abdomen and transform all the abdominal segments into a T2 identity.

i. The other homeotic genes in the same cluster as Ultrabithorax are abdominal A and abdominal B
   i. abdominal A regulates the 2nd to 5th abdominal segments
   ii. abdominal B -- 5th abdominal to 8th abdominal

   These three genes act in combination to repress other thoracic-specific genes such as Distalless which specifies the legs.

j. Since their discovery in flies, the homeotic genes were found to have genes in most organisms that are related via the highly conserved HOMEODOMAIN.

k. Lewis thought of the idea that the homeodomain family has undergone gene duplication and divergence during evolution. Humans also have homeotic genes that are expressed at different places along the A-P axis. In mice these genes are called HOX GENES and have some amazing properties in common with fly homeotic genes (more in mouse lecture). Hox3.1 example.

**Winner takes all: Notch and the competition to be a neuron**

1. Remember that the ectoderm goes on to form both the epidermis and nervous system.
   a. In fruit flies nervous system individual cells ingress to become neuroblasts.
   b. Ventral most ectoderm= neurogenic ectoderm.
   c. About 30% of the cells ingress to form neuroblasts, the rest go on to form ventral epidermis

2. What decides which cell takes which fate? Clue- in flies mutant for either the Notch gene or the Delta gene, **all ventral ectoderm cells forms neuroblasts**. Also in mutants big brain and mastermind: all cells moved in and became neurons, no skin.
   a. **Hypothesis #1-** All cells in region have potential to be neuroblasts.
      i. In the ectoderm a cell would first become a neuroblast by CHANCE, but would then send signal to neighbor- "I'm a neuroblast- you can't be one!"
   b. **Hypothesis #2--Notch & Delta act in the signaling process-** If mutant, signaling disrupted & all cells become neuroblasts.
      i. There are several genes with same phenotype as Notch & Delta ie. all neuroblasts, no epidermis
   c. **Hypothesis #3—they encode different parts of signal transduction pathway.**

3. Are all of these hypotheses true? What part of signaling pathway do Notch and Delta encode?
   a. **Experiment #1-** Identify products of Notch and Delta genes.
      i. **Notch** encodes transmembrane protein with large extracellular & large intracellular domains. Could it be the receptor for the signal??
      ii. **Delta** encodes transmembrane protein with large extracellular & very small intracellular domains. Could it be a membrane-bound signal??
b. **Experiment #2**- Do Notch and Delta physically interact? (like a lock and key fit)
   i. Cells in culture. Express Notch on surface of one cell and Delta on surface of other cells, and label cells with different colors. Mix together- do they sort out or stick together? Who sticks to who?
   ii. Result- Notch-expressing cells stick to Delta-expressing cells. **Therefore, the proteins interact!** Consistent with idea that one is ligand, one is receptor.

c. **Experiment #3**- Notch and Delta -- which is signal and which is receptor? Can distinguish these with a simple test:
   i. Case A. Make animal where most cells are wildtype, small group of cells is mutant. Mutant cells lack the ability to make signal while the wildtype neighboring cells make signal. Result: The **mutant cell is rescued by neighbors.**
   ii. Case B. Surround small group of mutant cells lacking receptor with a large number of neighboring cells making signal (and possessing receptor). Result: **mutant cell is not rescued by neighbors since unable to sense signal.**
   iii. Try the above with Notch and Delta mutant cells to distinguish between signal (Case A) and receptor (Case B). The results:
      1. Delta mutant cells rescued by neighbors. Therefore, Delta must be the signal
      2. Notch mutant cells NOT rescued by neighbors. Therefore, Notch must be the receptor
   iv. **Where are the genes expressed?** Notch on epidermis-fated cells, and Delta on new neuroblasts. These patterns further support the model that Delta is the signal produced by new neuroblasts, and that Notch is the receptor on the other cells that receives the signal to remain epidermal.
   v. When Notch and Delta bind, proteolytic cleavage cleaves the cytoplasmic domain of Notch, which then goes to the nucleus, binds a TF and turns on genes.
   vi. This system is conserved in our bodies in situation where cells make a binary decision, and is an example of one of the signal transduction pathways used over and over. Ie. decision whether to be a helper T-cell or a killer T-cell is mediated by Notch.
   vii. Inappropriate activation of Notch pathway plays a role in lymphoma or blood disease.