

Lecture 7. MCB 141. Compartments and wing patterning in *Drosophila*.

Reading: Scott Gilbert Developmental Biology 7th Edition, pgs 228-256; 499-502

1. We discussed how different combinations of gap repressor gradients produce 7 pair-rule stripes of eve expression. A similar logic is used to produce 7 stripes of hairy expression. However, in the case of Hairy, each stripe is shifted anteriorly relative to each eve stripe. Propose a model for these shifts. For example, why do the hairy and eve stripe 2 enhancers produce offset expression patterns?
2. Hairy augments eve expression in regions where the two stripes overlap. This results in the production of eve “minigradients”, with each eve stripe exhibiting peak levels of protein at the anterior margin and progressively lower levels in more posterior regions.
3. The Eve protein works as a repressor to produce differential patterns of ftz and odd-skipped (odd) expression. The anterior-most cell expressing each of the 7 ftz stripes activates engrailed in 7 pair-rule “lines” of expression since the Odd protein prevents the ftz activator from inducing engrailed expression. Peak levels of the Eve protein, at the anterior margin of each eve stripe, activates engrailed in 7 alternating lines of expression.
4. Together, the complementary eve and ftz pair-rule stripes produce 14 lines of engrailed expression: 3 in the posterior head, 3 thoracic, and 8 abdominal. The engrailed lines divide each developing segment into separate anterior and posterior compartments. These different lineages are retained throughout the life cycle of the fly. That is, all of the daughter cells of the Engrailed-expressing cells remain in the posterior compartment, whereas the cells lacking Engrailed remain in the anterior compartment.
5. The adult wing arises from an invagination of about 30 cells in the second thoracic segment, T2. The posterior 10 cells arise from the posterior compartment of this segment and therefore express Engailed. The anterior 20 cells define the anterior compartment of the developing wing.
5. After 20 hrs of development, the fly embryo hatches into a first instar larva. This grows for a day and molts into a second instar larva. A day later there is yet another molt and a third-instar larva is formed that grows and gets quite fat during a period of two days. The third instar larva finally forms a pupa (fly cocoon) and emerges into an adult fly about a week later.
6. All of the adult appendages of the adult fly, the legs, wings, antennae and eyes, are produced from thin sheets of cells called imaginal disks (NOT imaginary disks!). In third instar larvae the wing imaginal disk is composed of about 30,000 cells. The posterior 10,000 cells express Engrailed and define the

posterior compartment. The anterior 20,000 cells do not express Engrailed and define the anterior compartment.

7. Engrailed leads to the expression of Hedgehog, a signaling protein that we have previously discussed with respect to the activation of Nkx6 in ventral regions of the vertebrate neural tube. In *Drosophila*, Hedgehog is expressed throughout the cells of the posterior compartment. The protein is secreted but does not travel very far into the anterior compartment. At the site of entry, the Dpp gene is activated in a thin stripe of cells, about 3-4 cell widths, at the posterior edge of the anterior compartment.

8. Dpp is secreted and travels over long distances in the disk and forms a symmetric gradient emanating from the boundary between the anterior and posterior compartments. So, there is a signaling relay: the short-range Hedgehog signal triggers the production of a long-range Dpp signaling gradient. There is a lot of controversy regarding the mode of transport of the Dpp signal from its source at the compartment boundary. Some say that the Dpp protein diffuses through the extracellular matrix. Others believe that all of the cells of the wing disk send specialized filopodia, called cytonemes, and grab Dpp at its source of synthesis. According to this view, cells located near the source grab more Dpp than those located far away. Either way, diffusion or cytoneme-mediated transport, a broad Dpp signaling gradient is created across the width of the wing imaginal disk.

9. This gradient produces different thresholds of gene expression across the wing disk. Peak levels activate *spalt* at the compartment boundary, while intermediate levels activate *Omb* and the lowest levels activate *vestigial* throughout most of the disk. The Dpp signaling gradient also represses the target gene *Brinker*, which is restricted to the margins of the wing disk. *Brinker* encodes a transcriptional repressor that works in an opposing manner to the Dpp/Smad gradient emanating from central regions. I want you to consider how the Dpp gradient leads to different thresholds of *spalt*, *Omb*, and *Vestigial* expression. You should also have a sense of how Dpp leads to a reciprocal *Brinker* repressor gradient. Hint: think about the repression of Dpp by the Dorsal gradient in the early *Drosophila* embryo.