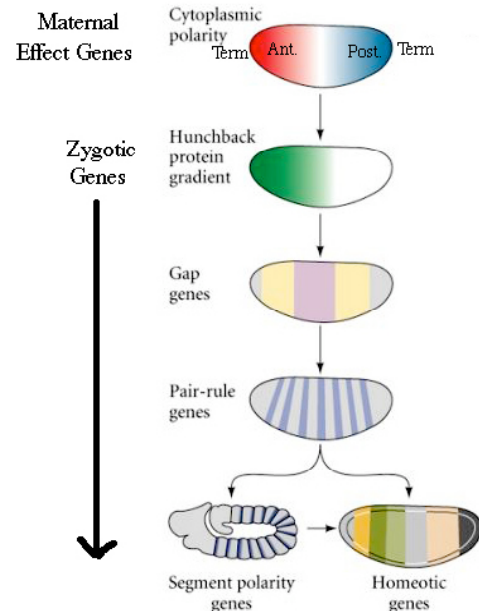


Modeling the Organism: The Cell in Development

Laboratory 6: Segmentation in *Drosophila*

Introduction

One of the key aspects of metazoan development is the specification of cell fate as a single cell generates a multi-cellular organism. Given the complexities of this process, it is amazing that evolution uses a remarkable variety of different mechanisms to pattern embryos, ranging from the highly stereotyped, lineage-dependent, mosaic development of *C. elegans* to the very plastic, cell-signaling-dependent, regulatory development of mammals. *Drosophila* and other insects use an intriguing variation in which a syncytial cytoplasm is structured with positional information in the form of transcription factor gradients; this information is then partitioned among many nuclei in a massive cytokinetic event called cellularization.



<http://courses.biology.utah.edu/bastiani/3230/DB%20Lecture/Lectures/Lec8FlyMaternal.html> , cached 060221

These information gradients are initiated during oogenesis, when the mother provisions the egg with the products of the Maternal Coordinate Genes (such as Bicoid, Nanos, Caudal). These establish the anterior-posterior and dorsal-ventral axes, and in turn effect the expression of the Gap Genes (Hunchback, Kruppel, Knirps) which define the anterior-middle-posterior of the embryo. Each nucleus enters a unique pattern of gene expression during cellularization as the Pair Rule Genes (Even-skipped, Hairy, Fushi Tarazu) are invoked to create 7 bands of gene expression along the embryonic axis, and these in turn activate the Segment Polarity Genes (Wingless, Hedgehog, Engrailed) and Homeotic Genes that define the identity of cells within each of the 14 segments of the embryo. Although further morphogenetic events such as gastrulation occur as the embryo develops into a larval form, as a result of this cascade of gene activation each cell of the embryo already has a unique position and fate.

In this week's lab, each pair of groups (you and the group across your lab bench) will receive three plates of *Drosophila* embryos labeled A-C. Each of these strains expresses a specific promoter - reporter gene fusion such as:

Hunchback-lacZ
Fushi tarazu-lacZ
Engrailed-lacZ

The *lacZ* gene used in these constructs is originally from *E. coli*. In the first lab of this quarter you studied *lacZ* (beta-galactosidase) regulation and expression under the control of the *lac* promoter in *E. coli*. Today we will use the same open reading frame under the control of promoters cloned from different *Drosophila* transcription factors as a tool to study patterning in the developing *Drosophila* embryo. In order to do this, we will first harvest a mixed population of embryos (so as to catch a few that are at the right stage to be expressing the reporter construct) and then process them to localize *lacZ* expression using the colorimetric reagent X-Gal. X-Gal is very similar to the GUS substrate X-Gluc used in the *Arabidopsis* lab in being a

colorless substrate that is converted into a product that is colored and precipitates to mark the site of enzymatic expression.

Procedure

1. Egg collections. Eggs are collected from apple juice-agar plates using a deionized water wash bottle and a P1000 pipetter or a paint brush. Transfer some embryos from each plate to a different well of a glass multi-well dish and examine them under the dissecting microscope. Notice the dorsal respiratory appendages ("rabbit ears") at the anterior end of each embryo.

2. Dechoriation. In order to permit the staining solutions to enter the embryo, it is necessary to remove of the chorion or egg coat. Remember that bleach ruins clothes, so be careful!

Use a yellow tipped P100, carefully remove the water from around the embryos. Replace with 500uL 50% bleach, making sure that the bleach covers the embryos, and place on the shaker in the hood. Dechoriation should be complete in 5-10 minutes. The respiratory appendages will dissolve during this process, and you can check this under the dissecting scope. When they are gone, dechoriation is complete.

Remove the bleach with a P-100 and replace with 500 uL PBT (1x phosphate-buffered saline + 0.1% Tween-20, a detergent) and place the plate on shaker to wash the eggs for 5 minutes. Wash the embryos with a second change of PBT for and shake for another 5 minutes. What is the purpose of the Tween-20?

3. Fixation. (Caution: Wear gloves for this step and work in the chemical fume hood) Again using a P100, remove the PBT wash and replace with 400 uL glutaraldehyde-saturated heptane (the heptane fixative is the upper phase; avoid the glutaraldehyde lower phase). **Heptane** permeabilizes the embryonic vitelline membrane, while **glutaraldehyde** covalently cross-links proteins to preserve morphology. It is important not to fix embryos too long! You're trying to fix long enough to preserve morphology, but not so long that you destroy the β -galactosidase activity, so fix while gently shaking (in the chemical hood) for **4 minutes only!**

Pipette the fix away from the embryos and into the appropriate labeled waste container using the P100. Add 500 uL of PBT to the well and shake your depression slide gently (on shaker!) for 5 minutes. Remove the PBT into another waste container, replace with fresh buffer and shake again for 5 minutes. Repeat for a total of 3 washes.

4. X-Gal staining. Remove the PBT wash from your embryos and replace with 400 uL stain mix. Warm this in a 37° incubator for 5 minutes. To this add 20 uL of 4 ug/ml X-Gal solution. Cover your multi-well slide with Parafilm and incubate at 37°. The blue staining reaction should occur within 30-60 minutes. You can monitor the staining reaction by viewing your material under the dissecting scope; if the signal is weak, return the specimens to the incubator for further development.

Analysis and Interpretation:

1. For the reporter gene embryos, what proportion of the embryos showed the staining pattern? Why? Describe the reporter gene expression pattern(s) present in each batch of embryos. Make sketches of a few representative embryos that show good staining.

2. Determine which step in the developmental cascade shown on the previous page is represented by each reporter gene expression pattern in strains A-C . Using the identities of the reporter constructs given on the previous page and some external research, try to assign each to a specific strain.

Lab report: Each student will be responsible for submitting a one page lab "brief" describing the results from one of the following lab modules: (1) the *Arabidopsis* experimental module (started two weeks ago); (2) the *Planaria* regeneration experiment (starting last week) or (3) the *Drosophila* patterning investigation (starting this week). Your brief should include some hand drawings of your results and will be due at the start of lab in the last week of the semester. Note that everyone is responsible for completing all three units and entering data and describing results in their lab notebook; however, you are only responsible for writing up one of these three experiments. Lab partners are free to write up the same or different modules, but each student should complete their own brief.