Experiment I Zygotic Gene activity, MBT and Gastrulation

Here we look at early embryos under halocarbon oil to follow the progressive changes in morphology between two and four hours of development. This can be done under a stereomicroscope without dechorinating, observing the embryos directly on the agar filled Petri dishes on which the flies have laid their eggs. Resolution can be improved by transferring the embryos to a glass slide and using the bright field optics of a compound microscope. It is also possible to remove the chorion with bleach. These are improvements but they are not essential.

Stocks examined in these experiments

C(2L); C(2R) - Compound second chromosomes, generates 2L- halo embryos that fail to cellularize, and 2R- embryos that fail to gastrulate normally.

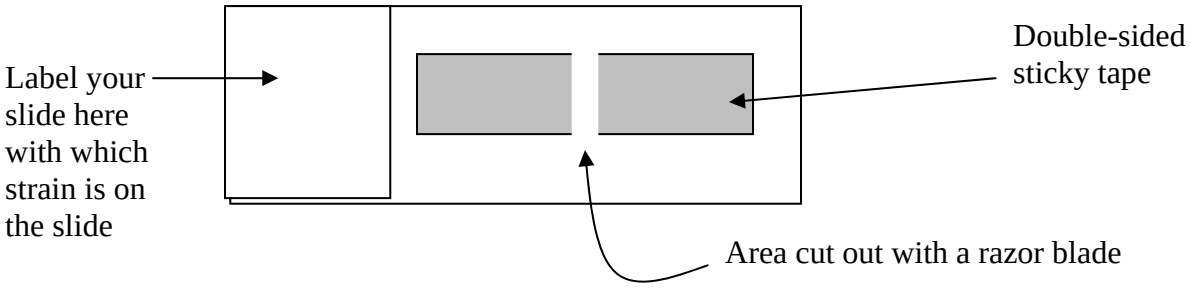
twi^{YD96} / CyO ; klarsicht – generates ¼ *twist* embryos (in a particularly photogenic klarsicht background)

fog^{4a6} / FM7 ; klarsicht – generates ¼ *folded gastrulation* embryos (in a particularly photogenic klarsicht background)

Intellectually the interesting point are 1) that none of the embryos show any abnormalities until the beginning of cycle 14, even through the embryos from the compound cross are missing 20-40% of their genome, 2) that when defects occur they are discrete and recognizable and reflect the role of specific zygotic gene products in cellularization and gastrulation, 3) that the 2R- phenotype is initially almost the same as the twist mutant embryos (the wildtype allele of twi is located on that arm) but it is subtly different (why and how it is different is an interesting challenge) 4) fog is the best characterized downstream target of Twi in the mesoderm and yet its phenotype is not the same (again how and why not?)

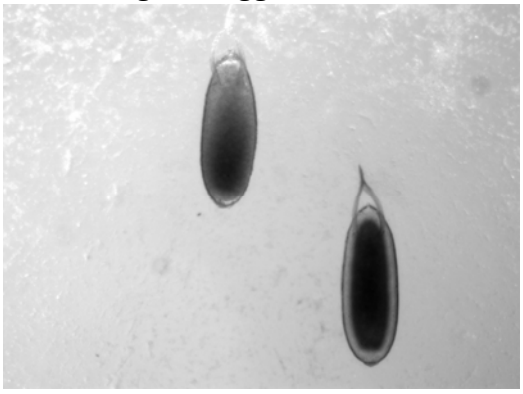
Preparation of living Drosophila embryos for examination using a bright field compound microscope illumination or epifluorescence microscopy

Prepare a glass depression slide. Place a piece of double-sided sticky tape horizontally on the glass slide. Use a razor blade to cut a vertical thin strip from the middle of the tape. Remove this strip of tape using the corner of the razor blade. Try not to leave jagged edges.



To obtain Drosophila embryos, adult flies are transferred to collection cups sit on petri dishes contatining a transparent agar egg laying medium. The eggs are laid on the surface of the agar and can be visualzed under a stereomicoscope with transmitted light. The eggs are covered bt two egg shell, the opaque outer chorion and the tranparant inner vitelline membrane

Transfer some halocarbon oil to the surface of the plate. The oil should coat the surface of the plate. The oil penetrates the chorion (the outer egg shell that surrounds the drosophila embryo)and makes it transparent such that you can now see through the egg shell and into the embryo. With light from the base of your microscope, the blastoderm stage embryos have a dark opaque yolky center and a clear uniformly thick surface layer containing the newly formed blastoderm cells. Unfertilized eggs and embryos in the very early mitotic divisions are uniformly opaque. In the earlier syncytial stages the clear surface layer is thinner and grades off into the yolk, at later gastrula stages the layer is no longer uniform and you should see the mesodermal and posterior midgut invaginations, as well as the cephalic furrow.



You can follow the progress of gastrulation and distinguish mutant and wild type embryos by the appearance of this layer. The blastoderm at the right is viewed from the dorsal side. For a lateral view, you can role the embryos on the agar so the right and left dorsal appendages both point up..

For higher resolution views, you can transfer the embryo to a drop of halocarbon oil on a glass slide for examination with bright field optics in a compound microscope.

The chorion can be removed by treating the embryo with bleach. Bleach does not remove the vitelline membrane. In a small petri dish soak a small piece of paper towel with bleach. The paper towel needs to be fully saturated but not floating in bleach. Transfer 15-20 embryos

to the paper towel. The bleach will remove the outer chorion (eggshell) that is around the embryo. Removing this will make the morphology even clearer when you have the embryos under the fluorescence microscope. The process of removing the chorion with bleach takes only a few minutes, and once removed the embryos will appear shiny when viewed under the dissecting microscope.

Pick up the piece of paper towel with the embryos using the forceps and transfer it to a fresh towel or the bench paper (embryo side up!) to blot away most of the excess bleach. Transfer the piece of paper towel to the glass slide and place it on top of the labeled area.

Add a drop of halocarbon oil to the channel you created with the tape on the glass slide and transfer the embryos from the paper towel to the halocarbon oil on the slide. The embryos will stick to the forceps if you dip the tip of the forceps into the halocarbon oil. Try to arrange them in a line rather than a clump in the middle. Slowly lower the coverslip down over the embryos allowing the oil to adhere to the coverslip slowly in order to keep air bubbles to a minimum. You may need to put a drop of oil at the edge of the coverslip in order to fill in any air pockets.

Embryos can be visualized with bright field optics under a compound microscope.

Experiment II Anterior / Posterior Patterning in Early Embryo

bcd^{E1} / TM3, Sb (=1X Bcd and 0X homozygotes)

EGFP-Bcd-4A; bcd^{E1} (=2X Bcd)

EGFP-Bcd-4A; EGFP-Bcd-10 bcd^{E1} (=4X Bcd)

It is possible to follow anterior posterior pattern in gastrulating embryos from these stocks, using halocarbon and stereomicroscopes (the same procedures used above). The interesting thing here is the position of the cephalic furrow that marks the boundary between the head and thoracic primordia in the embryo. The position of the furrow is shifted in response to Bcd dosage, and associated with this shift are the changes in the relative size of the head, thorax and abdominal primordia. Intellectually the interesting question is how the embryos compensate for these shifts in cell number.

Because the Bcd proteins are marked with EGFP it is also possible to visualize the Bcd gradient in living embryos, or in fixed embryos using antibodies to GFP. You can see the Bcd gradient using a standard epifluorescence microscope, but it is obviously more impressive in a confocal microscope.