

Developmental genetics in Drosophila: early patterning of the embryo

Reading: 8e: Chp 9: 253-278; 9e: Chp 6: 203-211

Learning Goals Be able to:

Interpret the phenotypes and interactions between the maternal effect egg polarity genes in patterning the early embryo

Relate the expression of the egg polarity genes (and their function) to the expression and function of the gap genes to explain the second level of patterning of the embryo

Predict outcomes on expression of various embryonically transcribed genes as a consequence of the loss of other genes that regulate them.

Application of the genetic approach to Drosophila development has been strikingly successful in elucidating the genetic control and molecular mechanisms of pattern formation. Drosophila now provides the most completely understood process of embryonic patterning, and many of the lessons learned from it apply to other embryos, both invertebrate and vertebrate. The patterning process involves progressive establishment of the A-P and D-V axis polarities, specification of broad domains of the body plan along the A-P axis, establishment of repeating segments along the A-P axis, and then specification of different segment identities, to give a segmented animal in which every segment is unique. We will work through these patterning steps in this and the next class. The first part of these notes (in brackets) is a complete explanation of Drosophila; we'll review it in class, and you may find it necessary for understanding genetic control of early development.

[Life cycle**Oogenesis**

In the Drosophila ovary, each oogonium (primordial female germ line cell) undergoes four incomplete mitotic divisions, to produce 16 cells all connected by cytoplasmic bridges. **One** of these cells becomes the oocyte, and the rest serve as *nurse cells*, which supply cytoplasmic macromolecular components to the oocyte as it grows and matures. Somatic *follicle cells*, **not** derived from the germ line, form an epithelial layer around each oocyte and accompanying nurse cells; they are connected to the oocyte by gap junctions, which allow exchange of small molecules only. They also may secrete macromolecules that interact with the oocyte cell surface or are taken up by endocytosis. The nurse cells and follicle cells are responsible for early interactions that determine localization of mRNAs in the oocyte.

Embryonic development

Cleavage and blastoderm formation. After fertilization, nuclei go through rapid rounds of division in the central, yolky region of the egg. These early divisions occur in a syncytium; the nuclei are not separated by cell membranes, and there is little transcription from the embryonic genome. During the eighth and ninth rounds of nuclear division, most of the nuclei migrate outward. A few nuclei move into the pole plasm at the posterior end of the egg at cycle 9 and become enclosed in cell membranes at cycle 10, forming the germ cells (pole cells). The somatic nuclei enter the cortical cytoplasm at the surface of the egg at the beginning of cycle 10. During the *syncytial blastoderm* stage, these somatic nuclei go through four more rounds of division, during which the cell cycle gradually becomes longer. At nuclear cycle 14, the cell cycle becomes much longer and the nuclei and their surrounding cortical cytoplasm are finally enclosed in membranes, forming the cellular blastoderm. Keeping in mind that the blastoderm in Drosophila is syncytial is critical for understanding how its patterning works.

MBT and gastrulation onset. Before the onset of cycle 14, the embryos go through the mid-blastula transition (MBT), marked by the initiation of bulk embryonic RNA synthesis and an asynchrony of cell cycles with addition of variable G1 and G2 periods. The lengthening of the cell cycle appears to be governed by the

DNA/cytoplasmic ratio, as in *Xenopus*, and the lengthening of the interphase period will occur earlier if the nuclear cycle is arrested by drug treatment. Both cellularization and gastrulation require new RNA synthesis. Somatic cells begin to take on position specific differences in behavior almost as soon as they are formed at cellular blastoderm. For gastrulation to occur correctly, cells at specific positions must go through different, stereotyped changes in shape and behavior. Thus, by the end of cellular blastoderm, cells have committed to particular fates based on position in the embryo: due to the syncytial nature of the early embryo, different concentrations of the many transcription factors will specify the fate of cells at different positions along the A-P and D-V axes of the embryo. Gastrulation in *Drosophila* is superficially different from that of vertebrates, but does begin with a hollow spheroid of cells and end with three germ layers. Somewhat later, the neural tube is formed ventrally, from neurogenic ectodermal cells on either side of the midline and procephalic neuronal ectoderm in the head. An important class of cells, the *imaginal disc* cells that will form the adult, are set aside from the remaining ectoderm. Additional organogenesis occurs during the latter half of embryogenesis.

Larval development, metamorphosis, and adult morphology

The embryo hatches as a *first instar larva* or imago, in which each segment is marked by characteristic rows of bristles on the ventral side and hairs on the dorsal side. The cells that will form the adult are sequestered in 19 imaginal discs and a set of histoblast nests. The disc cells remain diploid (other larval tissues become polytenized) and divide so that the discs grow from about 50 to about 50,000 cells/disc during larval development. The larva undergoes two molts, to give second and third instars, followed by *pupation* and *metamorphosis*. The adult body arises from imaginal discs and histoblast nests during metamorphosis. Discs turn inside out to form adult ectodermal (cuticular) structures. Interestingly, the cells that will form the imaginal structures are segmentally determined at the cellular blastoderm stage in the embryo. Damage or removal of cells from the cellular blastoderm results in structures missing in the larva or adult, allowing the construction of blastoderm fate maps. Cells from the blastoderm, when transplanted, develop autonomously into adult structures characteristic of the cell's position of origin.]

The developmental genetics exploratory approach to *Drosophila* early embryogenesis:

During oogenesis, the *Drosophila* oocyte acquires both an A/P and a D/V polarity. Initial clues that there were maternal determinants important for A-P patterning were due to classic embryological removal and replacement types of experiments. Removal of cytoplasm from the anterior end resulted in an embryo without a head, while transplantation of anterior cytoplasm into the posterior end of a normal embryo resulted in an embryo with two heads. What genes were involved?

Systematic, large scale mutant hunts for both maternal and nonmaternal-effect (zygotic) embryonic lethal mutations, begun in the early 1980's by Nüsslein-Volhard and Wieschaus, identified most of the genes expressed in the embryo that are involved in first establishing the polarity of the embryo, and then the segmented body pattern. They received the Nobel Prize for this work in the late 1990s. They found that in many cases, homozygous mutant embryos survive until the end of embryogenesis and secrete a cuticle, but die without hatching from the eggshell. Nüsslein-Volhard and Wieschaus looked at the unhatched, dying embryos and identified those with defects in polarity or patterning, separating them into four classes of genes that act sequentially to pattern the embryo: *egg polarity*, *gap*, *pair-rule*, and *segment polarity* genes.

Subsequent understanding of these genes and their functions resulted from 1) positional cloning and sequencing, 2) determining spatial patterns of normal expression, either by *in situ* methods or using reporter constructs, 3) determining effects of mutations in other genes on these spatial patterns, and 4) detailed functional analysis of promoter and enhancer elements controlling these genes.

The hierarchy of segmentation genes

To summarize conclusions at the outset, the three classes of segmentation genes (*gap*, *pair rule* and *segment polarity*) function hierarchically. All are required to be expressed in the embryo, before or during cellular blastoderm stage, for normal patterning.

Expression of the *gap* genes, which define broad overlapping regions along the A-P axis, is regulated by products of the maternally expressed *egg-polarity* genes (bicoid and nanos; discussed below). In addition the *gap* genes, which also encode transcription factors, regulate their own and each other's expression. The *gap* genes end up expressed in broad domains at the cellular blastoderm stage.

The next phase of patterning begins the process of “segmentation”: the cells of the embryo are separated into repeated units. This is maintained through the larval stages, and each of these regions contributes to a segment of cells that are recognizably different from each other in the adult fly. The *gap* gene products regulate the expression of three *primary pair-rule genes*, which in turn regulate at least five *secondary pair-rule genes*, in different overlapping repeating patterns or “stripes” corresponding to pairs of segments.

The products of the *pair-rule* genes, also transcription factors, regulate expression of *segment-polarity* genes in each parasegment. Some of the *segment-polarity* genes encode transcription factors, but others encode proteins involved in cell signaling. They function to establish and maintain sharp boundaries between the segments as cell proliferation and gastrulation proceed. Finally, different Hox genes expressed in each of these regions determine the identity of the cells.

The sequential functions of these genes in patterning are summarized briefly below.

A-P polarity is established in the egg before fertilization by *egg polarity* gene products that function both in the germline itself (the egg and the nurse cells that synthesize its contents) and the surrounding follicle cells of the ovary. Two egg polarity gene products (Bicoid and Caudal) become distributed as opposing gradients in the syncytial embryo. Bicoid acts both as a transcription factor (activates the *gap* gene *hunchback*), and as a translational repressor of *caudal*. *Bicoid* mRNA starts off localized at the anterior while *caudal* mRNA is uniformly distributed. As soon as Bicoid protein is made, it establishes the gradient of Caudal (high at posterior, low at anterior) due to its repression of *caudal* translation. Another egg polarity gene, *nanos* helps to further the A-P nature of the embryo—its mRNA is deposited at the posterior of the oocyte, and then Nanos protein inhibits the translation of the *gap* gene *hunchback*. *Bicoid*'s action as a transcription factor for *hunchback* (embryonically) adds to the establishment of the gradient of *hunchback* (high at anterior, low at posterior).

The *gap* genes encode transcription factors that define different broad regions along the A-P axis. Most *gap* genes are transcribed embryonically (e.g. *Krüppel*, *knirps*), but a few (like *hunchback*) are transcribed both maternally and then again embryonically. The egg polarity gene products described above act as morphogens in the syncytial embryo, repressing translation and activating transcription in specific regions along the A-P axis, thus specifying different identities for broad regions of the embryo. The *gap* genes also encode transcription factors, and they repress each other's domains of expression so that they do not overlap by the time the embryo cellularizes at the end of the 14th cell cycle (note that embryonic expression has already begun before cellularization).

We will address the later elaboration of the repeating segmental pattern and specification of segment identities in the next class: these processes involve the pair rule genes and the segment polarity genes.

How does one determine function of genes before their protein products have been completely characterized? Mosaic analysis. [Note, this section tells you how mosaics are made in *Drosophila*, but you do not need to know those details, only be able to think about results of mosaic analyses].

Mosaics in flies (where the embryonic cell lineage is not known) are made differently than in worms (where the lineage is known). In this case, scientists take advantage of the unusual fact that chromosomal homologs pair during mitosis (normally this occurs only during meiosis). As a result, somatic (mitotic) recombination between homologs occurs spontaneously at a low frequency, which can be increased by X-irradiation. To create a mosaic, embryos heterozygous for a recessive mutation in a gene of interest are irradiated, resulting in an occasional cell that will divide to give adjacent homozygous wild-type cell and a homozygous mutant cell. As these cells divide, they give rise to a “clone” of wild-type cells and a clone of mutant cells (see the excellent diagram from a different textbook included in the ppt).

Because the goal of the scientist is to compare genetically wild type cells with genetically mutant cells and look at their phenotype (do they differentiate into their normal fate or not), a visible marker is closely linked to

the recessive mutation of interest. Then, after irradiation of a heterozygous larva the cells that are homozygous mutant will be visibly marked as genetically mutant. To determine if the gene product of interest works in a cell-autonomous (within the cell that has produced the protein) or cell non-autonomous manner (product acts in an adjacent cell), cells are analyzed along the border of the patch. If a genetically mutant cell ALWAYS has a mutant phenotype, then the gene product must act within the cell (cell-autonomous:: the phenotype of each cell is determined by its own genotype). If a genetically mutant cell is near non-mutant cells, and is phenotypically wild type, then the gene product must act non-autonomously (the phenotype of a cell can be determined by its neighbors' genotype). As we have already discussed, genes that encode receptors, downstream signaling components, and transcription factors act cell-autonomously, while genes encoding ligands for intercellular signaling act non-autonomously.

Review Questions

- 1) What is the overall strategy of pattern formation in the *Drosophila* embryo, and the four classes of genes that contribute to this process?
- 2) What are the general functions of egg polarity genes? Are they transcribed maternally or in the embryo?
- 3) How do *bicoid*, *hunchback*, *nanos* and *caudal* work in the A-P patterning of the early embryo?
- 4) What is the order in which the different sets of segmentation genes are activated in the embryo, and how do they work, in general?
- 5) What roles in patterning do the *gap* genes play? What kinds of proteins are they, and how is their transcription controlled?
- 6) With reference to the functions of egg polarity and gap genes, how does a strip of the cells in the middle of the embryo express mRNA for the gap gene *Krüppel* ?