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Eric Wieschaus' Lecture Part 1:
Pattern Formation in Drosophila Embryos

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1. Keywords and Terms

Embryonic development, pattern formation, morphogen, transcription, morphogenesis, gastrulation, diffusion, cooperativity, Drosophila, GFP, degradation, confocal microscopy, 2-photon microscopy

2. Lecture Notes

Following fertilization the Drosophila embryo undergoes 13 synchronous rounds of nuclear division by the end of which zygotic genes are expressed in distinct patterns in the embryo. These genes regulate subsequent events in the embryo such as gastrulation. The pattern of zygotic gene expression is regulated by a set of maternally provided cues.

One such signal is the morphogen Bicoid, which forms an anterior-posterior nuclear concentration gradient in the embryo. This gradient arises from maternal bicoid mRNA deposited at the anterior pole of the egg. Synthesis of Bicoid protein at the anterior pole, and diffusion from this site of production, results in the observed graded distribution of Bicoid. Nuclei can distinguish their position in the embryo based on the concentration of Bicoid. Some genes respond only to high concentrations of Bicoid in nuclei, as is found close to the anterior pole. Further from the source, the concentration of Bicoid declines and different genes are transcribed in response to lower concentrations of Bicoid. It is believed that the affinity of Bicoid for DNA binding sites determines this concentration dependent gene expression.

The genes that are regulated by Bicoid are called gap genes and encode transcription factors themselves. Cells expressing a certain combination/subset of these genes adopt a given fate (fate determination by combinatorial gene expression). For example, nuclei/cells at the anterior that were exposed to high concentrations of Bicoid, express the gap gene orthodenticle which confers a head fate onto these cells.

The activation of Bicoid target genes and the expression of most zygotic genes takes place at the mid blastula transition (MBT). In addition to the activation of the zygotic genome, maternally deposited mRNAs, such as bicoid mRNA, are degraded; in other words, the embryo switches from maternal to zygotic control of development. One consequence of the patterned expression of these genes is the correct spatio-temporal order of the morphogenetic movements such as ventral furrow formation.

3. Recommended Reading

1. Scott F. Gilbert. Developmental Biology 8th edition. Sinauer Associates Inc. 2006.

   This is an excellent textbook highlighting the seminal work in the field of developmental biology.

4. Review Questions

1. Bicoid is a morphogen that exists only in flies. Can we still learn something from studying a process in such an exotic animal? How would you convince others that your research in flies is important?
2. The aim of a majority of the research that is being conducted is to understand human biology. Why then do people use flies, yeast, worms, mice etc to investigate those processes? What is a model organism?

   If you had to identify the ideal model organism (for your research) which criteria would you chose?
3. What are the advantages of studying a morphogen in fly embryos rather than say mouse embryos?
4. How do the early embryonic cell cycles differ from the later ones? How long do they last? Are nuclei separated by cell membranes? What is a syncytium?
5. What is the mid blastula transition (MBT)? What events occur at the MBT? When does the genome of the mother matter and when that of the embryo? Can you name one gene that affects development only when mutant in the mother?
6. Following cellularisation, cells undergo morphogenetic movements in a stereotyped manner. How is this controlled?
7. Wieschaus discusses how spatial and temporal patterns of gene expression are established. Can you summarize the steps leading to that pattern?
8. Bicoid is a morphogen. What is the definition of a morphogen? Do you know other morphogens (in other animals)?

5. Answers to Review Questions

1. Many molecules/processes are conserved and are better studied using an experimentally tractable system. For example, many of the genes that we now know are involved in causing cancer in humans were first identified, and the principles worked out, in flies (one such gene is wingless, called wnt in vertebrates).
2. People use flies, worms etc (besides the obvious ethical reasons) because these systems are experimentally more easily approachable. For example, it is easy to

make a GFP fusion protein, express it in flies and follow these molecules by live imaging. A model organism is a species that is extensively studied to understand particular biological phenomena, with the expectation that discoveries made in the model organism will provide insight into the workings of other organisms. The following are a few examples of points worth considering when choosing a model organism: ease with which animals can be kept and raised; cost of animal maintenance; number of animals/embryos; time of development; methods available.

3. Mouse embryos develop in utero and are therefore not accessible to live imaging. Additionally, fly embryos develop much more quickly

4. The early embryonic cell cycles last about 9 minutes slowing down as the embryo nears cell cycle 14. Cycle 14 lasts about an hour during which time the zygotic genome is activated and cell membranes grow to separate nuclei from each other. The Drosophila embryo is special in that nuclear division is not followed by cell division. In fact nuclei divide within one cell, the egg, which is referred to as a syncytium. In cell cycle 14, membranes finally start separating the ~6000 nuclei. Once cellularisation is complete, the embryo undergoes a series of morphogenetic movements (gastrulation).

5. At the MBT the zygotic genome is activated (transcribed) and maternal mRNA is degraded. Thus the embryo begins to control its own development. One gene that affects development only when mutant in the mother (and not when the embryo or the father is mutant) is bicoid. There are several other genes like this for example oskar, torso and dorsal to name just a few.

6. This is controlled by a set of zygotically expressed genes. These genes are expressed in a distinct spatial and temporal pattern. This pattern itself is controlled by bicoid and other maternal genes (oskar, torso and dorsal).

7. The initial cues are provided by the mother who deposits mRNA encoding Bicoid (and others like torso, dorsal and oskar) in the egg. Because the mRNA for Bicoid is localized, a gradient of Bicoid protein forms providing the embryo with positional information. In other words, symmetry is broken. This gradient leads to the expression of target genes. Because these genes respond to different concentrations of Bicoid they are expressed in different domains.

8. A morphogen is a molecule (a protein or any other biologically active substance) that forms a concentration gradient and induces target genes in a concentration dependent manner. Often the fact that the morphogen has to act directly at a distance (and not through a relay mechanism) is emphasized.

6. Discussion Questions

1. Zygotic transcription begins at the mid blastula transition (MBT). What mechanisms can you imagine that prevent transcription before cycle 14? At what rate are genes transcribed? How long is interphase in pre-cycle 14 embryos? How could you test the idea that the physical length of a gene determines when it is transcribed?
2. The first genes to be expressed at the onset of zygotic transcription at the mid blastula transition (MBT) are a group of genes called gap genes which includes hunchback and krueppel. These genes are expressed in relatively broad domains. Subsequently, the pair-rule genes (e.g. paired, runt, even-skipped) are expressed in stripes that are 4 cells wide. How is this pattern achieved? How is the pattern refined or, in other words, how does the broad expression pattern of the gap genes give rise to the expression pattern of the pair-rule genes? What does combinatorial signalling mean? Is the time at which the pair-rule genes are expressed important? What events or cell behaviours are regulated by the pair-rule genes?

7. Answers to Discussion Questions

1. There could be a timing mechanism that is coupled to the transcription machinery. For example, some maternally deposited factor could become depleted and once it is below a certain threshold (which requires a certain time) it would trigger transcription. Another possibility is that the length of interphase in pre-cycle 14 embryos is too short to allow genes to be transcribed (or for transcription to be completed). Genes are thought to be transcribed at a rate of ~1kb per minute. Interphase in pre-cycle 14 embryos is ~ 5min. It follows that the maximum length of a transcribed unit could be 5kb if (and this is unlikely to be the case) the whole interphase period could be used for transcription. To test this idea, one could experimentally increase or decrease the length of a gene. The onset of transcription of this artificial gene should be delayed (if its physical length is increased) or be premature (if it is shorter). (see also assignment).

2. The pattern of gap gene expression is determined by the maternal genes bicoid, torso and nanos. The gap genes are transcription factors themselves and induce the

expression of downstream genes, the pair-rule genes. Some of the pair-rule genes respond only to a combination of the gap genes (combinatorial signaling) and therefore are expressed only in cells where two gap genes occur (e.g at the boundary between hunchback and krueppel). This way the initial broad pattern is refined. Some of the events or cell behaviours that are controlled by these genes are cell shape changes that cause morphogenetic movements like gastrulation. It is not only important to trigger these cell shape changes at the right time but also in the right place.

8. Explain or Teach These Concepts to a Friend

One fundamental question in developmental biology is how naïve tissue is provided with positional information. Explain what a morphogen is and how it provides a solution to the problem of positional information.

9. Research the Literature on Your Own

1. Rothe, Pehl, Taubert & Jackle. Loss of gene function through rapid mitotic cycles in the Drosophila embryo. Nature 359, 156-159, (1992).
2. Rothe, Pehl, Taubert & Jackle. Loss of gene function through rapid mitotic cycles in the Drosophila embryo. Nature 359, 156-159, (1992).
3. How is the timing of the expression of the genes knirps and knirps-related regulated? Could this be a general mechanism by which timing of gene expression is regulated?