

Lecture 3. MCB 141, S15. *Drosophila* embryo and segmentation.
Reading: Chapter 17, *Molecular Biology of the Gene*, 7th Edition.

1. The *Drosophila* embryo exhibits the most rapid nuclear divisions seen for any animal system, with peak speeds of a division every 5-7 minutes. Following fertilization, the oocyte and sperm pronuclei fuse and undergo a series of divisions without cytokinesis (cell divisions).

2. The first 7 divisions occur within the central yolk regions, while the next 3 divisions occur during the movement of the nuclei to the periphery. An hour after fertilization the embryo is composed of about 800 nuclei forming a monolayer around the periphery of the egg. During the next 30 min the nuclei undergo another 3 rounds of division to form nuclear cleavage cycle 14 embryos containing roughly 6,000 nuclei. These are referred to as syncytial blastoderm stage embryos.

3. During a one-hour period, from ~1.5 to 2.5 hrs following fertilization, cell membranes are laid down between neighboring nuclei to form cellular blastoderm embryos. The fate map of the adult fly is established during this one hour interval. For example, classical grafting experiments indicate that nuclei are totipotent prior to nc14, but during this period they become committed to following specific pathways of development.

4. Highly localized patterns of gene expression are established during cellularization, including localized stripes of expression that foreshadow the subdivision of the embryo into a repeating series of body segments. These stripes depend on localized maternal determinants already present in the unfertilized egg: bicoid and nanos. The bicoid mRNA is localized at the anterior tip of the egg and embryo, while nanos is localized to posterior regions.

5. The bicoid mRNA serves as a localized source of a regulatory gradient, with peak levels in anterior regions and progressively lower levels in central regions of the early embryo. The Bicoid protein contains a homeodomain that mediates binding to specific DNA sequences: TAATCC. Hunchback is one of the first zygotic target genes to be activated by the Bicoid gradient. This activation depends on an enhancer DNA located immediately upstream of the Hunchback promoter.

6. The enhancer contains three optimal Bicoid binding sites, with each site containing the flanking CC residues. These three sites allow both high and low levels of the Bicoid gradient to activate Hunchback transcription in the anterior half of the embryo. Residual levels of the gradient in the posterior half of the embryo are insufficient to activate transcription.

7. Hunchback is activated by Bicoid in the anterior half of the embryo and repressed by Nanos in posterior regions. Hunchback is also expressed during oogenesis, resulting in maternal mRNAs that are distributed throughout the egg and early embryo. Nanos inhibits the translation of these Hunchback mRNAs in posterior regions. This repression is essential for the normal patterning of the embryo, in that embryos lacking Nanos exhibit severe defects including the loss of abdominal body segments.

7. Hunchback is a so-called gap gene. It is important for patterning the future head and anterior thorax of the developing embryo. There are additional gap genes required to pattern the remaining regions. For example, the gap gene Knirps is required for the

development of anterior abdominal segments. It exhibits a pattern of expression that is complementary to the Hunchback pattern. These reciprocal patterns of Hunchback and Knirps depend on cross-repressive interactions.

8. The Bicoid gradient also activates the gap gene Giant in anterior regions of the embryo. Giant is activated only by high levels of the gradient and therefore exhibits a more restricted pattern of expression than Hunchback. The differential activation of Giant and Hunchback by Bicoid could arise from differences in the affinities of Bicoid binding sites in the two enhancers.

9. Giant represses Kruppel and restricts it to central regions of the embryo. So, there are two pairs of cross-repressive gap genes: Hunchback/Knirps and Giant/Kruppel. These four sequential gap genes produce many stripes of pair-rule gene expression underlying segmentation.

10. Consider one of the eight pair-rule genes, even-skipped or eve. It is regulated by 5 separate enhancers, which are located upstream and downstream of the transcription unit. Each enhancer is ~500 bp in length and generates one or two stripes of gene expression. The eve stripe 2 enhancer is located 1 kb upstream of the transcription start site. It contains 5 Bicoid binding sites and 1 Hunchback site that activate expression throughout the anterior half of the embryo. But the enhancer also contains binding sites for Giant and Kruppel, which repress the enhancer and thereby establish the borders of the stripe.

11. This combination of broad activation and localized gap repression is seen for all of the stripes. The eve 3+7 enhancer is activated by ubiquitous activators, while the borders are formed by Hunchback (anterior 3 and posterior 7) and Knirps (posterior 3 and anterior 7). The eve 4+6 enhancer exhibits a similar mode of regulation, except that low levels of Hunchback are sufficient to form the anterior stripe 4 border and posterior stripe 6 border. However, higher levels of the Knirps repressor are needed to repress the posterior stripe 4 border and anterior stripe 6 border, as compared with the 3+7 enhancer.

12. It is possible that the differential regulation of the 3+7 and 4+6 enhancers depends on different numbers of Hunchback and Knirps binding sites. The 3+7 enhancer contains just a few Hunchback sites and many Knirps sites, and conversely, the 4+6 enhancer contains many Hunchback sites and just a few Knirps sites.

13. The different eve stripe enhancers function independently of one another due to short range repression. Gap repressors inhibit only the activities of the enhancers to which they are bound. For example, the eve stripe 2 enhancer is repressed in central regions of the embryo due to binding by the Kruppel repressor. Kruppel bound to the stripe 2 enhancer does not interfere with the expression of the 3+7 enhancer in central regions.